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IMPROVED HEART FAILURE CARE ORGANIZATION INCLUDING STUDIES OF DIAGNOSIS, RISK PREDICTION AND BIOMARKERS IN HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Improved heart failure care organization including studies of diagnosis, risk prediction and biomarkers in heart failure with preserved ejection fraction.
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Моим родителям.

To my parents.

Climb Mount Fuji,
o snail,
but slowly, slowly.
Kobayashi Issa (1763-1828)

ABSTRACT

Background: Heart failure (HF) is a prevalent condition with low quality of life, high morbidity and mortality, and high societal costs. HF can be divided according to left ventricular ejection fraction (EF) into HF with reduced (HFrEF) or preserved EF (HFpEF). Diagnosis of HFpEF is more complex than for HFrEF. Evidence-based treatment is well defined for HFrEF, but not proven for HFpEF. Therapy implementation in HF is poor and better organization of HF care could impact therapy and outcome. Biomarkers may fill knowledge gaps in the pathogenesis of HFpEF, and potentially be used to discriminate between HFpEF and HFrEF.

Aims: To study 1) prevalence and prognostic importance of diagnostic echocardiographic variables in patients with suspected HFpEF, 2) effects of introducing a comprehensive HF care program in a large urban region, 3) circulating extracellular vesicles (EVs) as biomarkers in HF. 4) if biomarkers for myocardial fibrosis and inflammation differ between HFpEF and HFrEF.

Methods and results:
Paper I. HFpEF patients (n=356) were included after a presentation with acute HF, clinical signs of HF, elevated natriuretic peptides and LVEF ≥45%, and studied for long term outcome. Diagnosis of HFpEF was assessed according to the 2016 ESC (European Society of Cardiology) Guidelines and confirmed in 76-94% of patients. We identified two independent predictors of prognosis: moderate or severe diastolic dysfunction, or ≥4 abnormal diastolic echocardiographic variables (both p <.01).
Paper II. A standardized program (4D HF) for optimization of HF management was implemented in Stockholm County 2012-2017. Yearly visits to the HF clinics increased 3.4 times from 3,372 to 11,527, and dispensed evidence-based HF medications increased (p<.0001). These effects were associated with lower numbers of admitted HF patients (n=35,880; decrease by 13-20%/10^6 inhabitants; p <.0001) and lower adjusted 1-year all-cause mortality or HF readmissions (HR 0.98 per year, CI 0.97-0.99, p <.0001).
Paper III. Patients undergoing elective coronary artery bypass graft surgery (n=81) were included and divided in three groups: HFpEF, HFrEF and Normal LV function. Blood samples from coronary sinus, radial artery, and right atrium were analysed for extracellular vesicles (EVs) in plasma. EVs coexposing Connexin-43 and Caveolin-3, or Connexin-43 and Troponin T showed significant transcoronary concentration gradients, with the highest levels in HFrEF patients. EV levels correlated with various HF related variables. Further studies should be performed to assess EVs as biomarkers in HF.
Paper IV. Patients with new-onset HF (n=247) were divided into HFpEF and HFrEF. Blood samples were analysed for biomarkers of fibrosis and inflammation. In HFpEF, collagen degradation (CITP), and inflammation (VCAM-1) were increased, and collagen cross-linking (CITP:MMP-1 ratio) reduced compared to HFrEF (p <.05). CITP was an independent discriminator of HFpEF vs HFrEF (OR 1.15 CI 1.03 - 1.28).

Conclusions: The use of 2016 ESC HF guidelines for diagnosis and risk prediction in HFpEF is strongly supported. Implementation of a Guideline-based HF management program is associated with improved health care quality and patient outcome. New biomarkers such as EVs originating from the myocardium, and markers of fibrosis and collagen degradation revealed differences between HFpEF and HFrEF which should be further explored..

SAMMANFATTNING *(på svenska)*

Bakgrund
Hjärtsvikt (HF) har hög mortalitet och morbiditet, är vanligt, med dålig livskvalitet för patienten och med höga samhällskostnader. Två typer av HF har definierats, vilka baseras på ejektionsfraktionen i hjärtats vänstra kammare (LVEF): hjärtsvikt med sänkt (HFrEF) respektive bevarad (HFpEF) EF. HFpEF saknar evidensbaserad behandling, till skillnad från HFrEF. Stora kunskapsluckor finns för patofysiologi vid HFpEF, och biomarkörer för genes, diagnostik och prognos vid HFpEF. Hjärtsviktsviktvårdens organisation och dess påverkan på sjukdomens utfall är ofullständigt belyst. Vi har studerat olika aspekter av diagnostik, både med ekokardiografi och med hjälp av biomarkörer i blodet. Vi har också studerat effekter av införandet av ett omfattande förbättringsprogram för hjärtsviktsvård i Region Stockholm.

Delarbete I
I studien inkluderades 356 patienter med nydebuterad akut hjärtsvikt; inklusionskriterier var kliniska sviktecken, förhöjd natriuretisk peptid och LVEF ≥45%. Vi bedömde i enlighet med Europeiska kardiologföreningens riktlinjer huruvida patienterna hade HFpEF. Denna diagnos kunde bekräftas i 76-94% av fallen. Vidare graderade vi den diastoliska hjärtfunktionen. Vi identifierade två oberoende prediktorer för sämre prognos: måttlig eller uttalad diastolisk dysfunktion samt förekomst av flera (≥4) avvikande ekokardiografiska variabler.

Delarbete II
Ett program för förbättrad sviktvård (4D HF) infördes i Region Stockholm 2012-2017. Programmet inkluderade en strukturerad utredningsprocess vid misstanke om HF med ekokardiografi och blodprover, inrättande av utökade sviktmottagningar vid sjukhusen, samt utbildningsinsatser gentemot primärvården. Vid utvärdering observerades att antalet besök på sviktmottagningar ökade, sviktorsakade sjukhusinläggningar minskade, och att mängden från apoteket uttagna sviktläkemedel ökade. Vi såg även en förbättring över tid i projektets kombinerade utfallsmått: död eller återinläggning för hjärtsvikt inom ett år.

Delarbete III
Sammanlagt 81 patienter som genomgick elektiv kranskärlskirurgi delades upp i tre grupper: HFpEF, HFrEF och Normal hjärtfunktion. Blodprover togs vid operationen från sinus coronarius, arteria radialis samt höger förmak. Vi fann transkoronara koncentrationsgradienter med betydligt högre nivåer i sinus coronarius för extracellulära vesikler (EV) som uttryckte Connexin-43/Caveolin-3 respektive Connexin-43/Troponin T; nivåerna var högst vid HFrEF. EV korrelerade med flera olika variabler för sviktdiagnostik. EV av kardiellt ursprung är intressanta biomarkörer vid HF men behöver studeras vidare.

Delarbete IV
Sammanlagt 247 patienter med nydebuterad HF delades upp i två grupper: HFpEF och HFrEF. Blodprover togs för analys avseende biomarkörer för fibros- eller inflammation. Nivåerna av CITP (kollagen-nedbrytning) och VCAM-1 (inflammation) var förhöjda i HFpEF-gruppen, samt kvoten CITP:MMP-1 förhöjd (lägre kollagenstabilitet). CITP var en oberoende prediktor för HFpEF vs HFrEF i vår studerade hjärtsviktspopulation.

Slutsats
Validiteten för internationella diagnostiska kriterier för HFpEF har styrkts. Ett standardiserat riktlinje-baserat program för optimering av sviktvården i en storstadsregion har genomgående positiva effekter på behandling och prognos. Extracellulära vesikler av kardiellt ursprung kan utgöra andra möjliga biomarkörer vid hjärtsvikt. En cirkulerande kollagen-nedbrytningsprodukt är en tänkbar biomarkör vid diagnostik av HFpEF.

LIST OF SCIENTIFIC PAPERS

I. Persson H, Donal E, Lund LH, Matan D, Oger E, Hage C, Daubert JC, Linde C; KaRen Investigators. Importance of structural heart disease and diastolic dysfunction in heart failure with preserved ejection fraction assessed according to the ESC guidelines - a substudy in the Ka (Karolinska) Ren (Rennes) study. *Int J Cardiol. 2019 Jan 1; 274:202-207*

II. Matan D, Löfström U, Corovic Cabrera C, Eriksson B.L, Ekström M, Hage C, Ljunggren G, Lyngå P, Wallén H, Malmqvist K, Linde C, Persson H. Reorganization of heart failure management and improved outcome – the 4D HF Project. *Scand Cardiovasc J. 2020 Sep 24:1-8.* doi: 10.1080/14017431.2020.1820075. Online ahead of print. PMID: 32969284

III. Matan D, Mobarrez F, Corbascio M, Ekström M, Hage C., Lyngå P, Eriksson M. J., Persson B, Linde C, Persson H, Wallén H. Extracellular vesicles as biomarkers in heart failure - a study in patients with HFpEF or HFrEF characteristics undergoing coronary artery bypass grafting. *Manuscript.*

IV. Matan D, Löfsjögård J, Ekström M, López B, Diez J, Kahan T, Linde C. Wallén H, Persson H. Circulating biomarkers for myocardial fibrosis in new onset heart failure in relation to preserved or reduced ejection fraction: Results from a pre-specified interim analysis of the PREFERS study. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ACEI	angiotensin converting enzyme inhibitor
ACS	acute coronary syndrome
AF	atrial fibrillation
ARB	angiotensin II receptor antagonist
ARNI	angiotensin receptor neprilysin inhibitor
ASE	American Society of Echocardiography
BB	beta blockers (beta-adrenergic receptor antagonists)
BNP	B-type natriuretic peptide
CABG	coronary artery bypass graft surgery
CCL	collagen cross-linking
CI	confidence interval (95% throughout this thesis)
CITP	carboxy-terminal telopeptide of collagen type I
CO	cardiac output
CRT	cardiac resynchronization therapy
DD	diastolic dysfunction (of the left ventricle)
DM	diabetes mellitus
DT	deceleration time
E/A	ratio of mitral E to A velocity
E/e´	ratio of mitral Doppler E velocity to average mitral tissue Doppler e´-velocity
e´	mitral tissue Doppler e´-velocity
EACVI	European Association of Cardiovascular Imaging
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
ELISA	Enzyme-Linked Immunosorbent Assay
ESC	European Society of Cardiology
EV	extracellular vesicle
HF	heart failure
HFmrEF	heart failure with mid-range ejection fraction
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HR	hazard ratio
HT	hypertension
ICD	implantable cardioverter defibrillator
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th Revision

IHD	ischemic heart disease
IQR	interquartile range
IVRT	isovolumetric relaxation time
JVP	jugular venous pressure
LAVI	left atrial volume index
LBBB	left bundle branch block
LV	left ventricle of the heart
LVAD	left ventricular assist device
LVEF	left ventricular ejection fraction
LVMI	left ventricular mass index
MAP	mean arterial pressure
MDT	multidisciplinary team
MMP-1	matrix metalloproteinase-1
MPO	myeloperoxidase
MRA	mineralocorticoid receptor antagonist
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
OAC	oral anticoagulants
OMT	optimal medical treatment
OR	odds ratio
PICP	carboxy-terminal propeptide of procollagen type I
PKCα	protein kinase C alpha
PTX3	Pentraxin 3
QRS	an electrocardiogram complex comprising the Q-, R- and S-waves
RAAS	renin-angiotensin-aldosterone system
RAASi	renin-angiotensin-aldosterone system inhibitors
RCT	randomized controlled trial
SD	standard deviation
TnT	Troponin T
TR	tricuspid regurgitation
VCAM-1	vascular cell adhesion molecule-1

1 GENERAL INFORMATION ON THE THESIS

1.1 FLOW CHART

This thesis consists of four papers. Three of them (Papers I, III and IV) are parts of larger studies, as illustrated in Figure 1 and described in more detail in the Methods section.

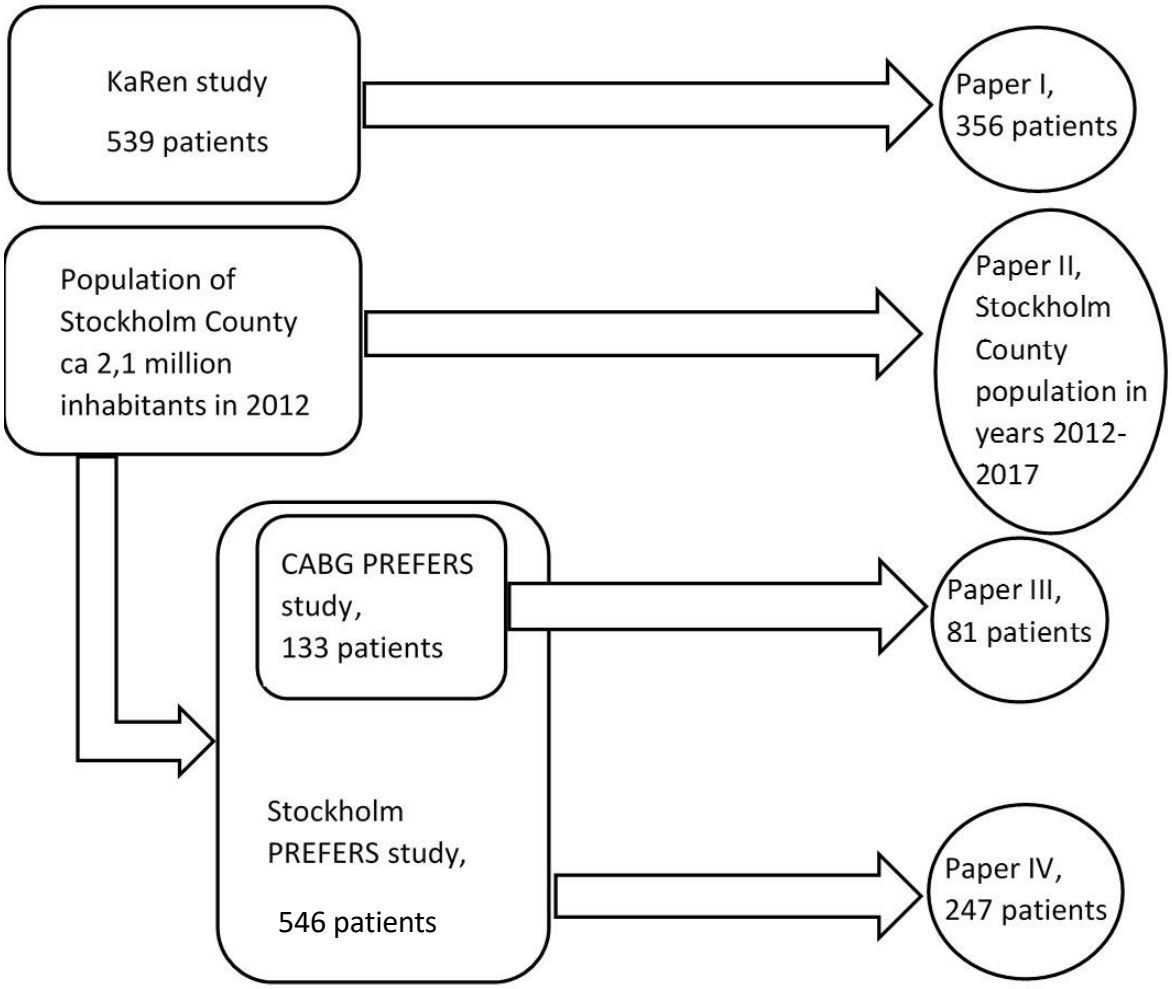

Figure 1. Flow-chart of the thesis.

1.2 INCLUSION AND EXCLUSION CRITERIA

The inclusion and exclusion criteria of the separate studies included in this thesis are presented in the Table 1.

Study name	Inclusion criteria	Exclusion criteria
KaRen	1. Acute HF presentation with signs and symptoms, according to the FC. 2. Elevated natriuretic peptides (BNP > 100 ng/L or NT-proBNP > 300 ng/L.). 3. LVEF ≥45%. All inclusion criteria must be present during the first 72 hours after the presentation.	1. Known HOCM, restrictive or infiltrative cardiomyopathy or isolated RV failure. 2. Constrictive pericarditis. 3. Severe pulmonary or renal disease. 4. CRT treatment. 5. High probability of CABG or TAVI within near future.
Stockholm PREFERS	1. New-onset HF diagnosed according to the current ESC guidelines. 2. Elevated natriuretic peptides (NT-proBNP >300 ng/L in ER or when the patient is admitted to the hospital, or >125 ng/L at HF clinic). 3. Age >18 years. 4. Written informed consent. 5. It must be possible to perform an echocardiography of sufficient quality according to a pre-specified protocol. 6. In HFpEF there must be LVEF ≥ 45% and E/e´ > 8. 7. In HFrEF there must be LVEF < 45%.	1. Cognitive disability. 2. Difficulties to understand Swedish language. 3. Anaemia (blood hemoglobin <90 g/L). 4. HF caused by valvular disease, RV failure, PAH, HOCM or infiltrative cardiomyopathy. 5. Various severe comorbidities that may impair the possibility for clinical assessment of HF.
CABG PREFERS	1. Patients undergoing elective CABG, with or without previous history of HF. 2. Written informed consent. 3. It must be possible to perform an echocardiography of sufficient quality according to a pre-specified protocol.	The same as for Stockholm PREFERS.

Table 1. The separate studies included in the thesis [1,2]. Abbreviations Table 1: BNP = B-type natriuretic peptide, CABG = coronary artery bypass graft surgery, CRT = cardiac

resynchronization therapy, E/e´ = ratio of mitral Doppler E velocity to average mitral tissue Doppler e´-velocity, ER = emergency room, ESC = European Society of Cardiology, FC = Framingham criteria, HF = heart failure, HFpEF = HF with preserved ejection fraction, HOCM = hypertrophic obstructive cardiomyopathy, HFrEF = HF with reduced ejection fraction, LVEF = left ventricular ejection fraction, NT-proBNP = N-terminal pro- B-type natriuretic peptide, PAH = pulmonary arterial hypertension, RV = right ventricle of the heart, TAVI = transcatheter aortic valve implantation.

2 INTRODUCTION

2.1 HISTORICAL PERSPECTIVES

HF was described already in the Antique world [3]. A better understanding of pathophysiology of HF was made possible by studies of the circulation by William Harvey in the 17th century and of hemodynamics by Otto Frank and Ernest Starling at the turn of the 19th into the 20th century [4,5].

During hundreds of years, the primary treatment for HF has been blood-letting (phlebotomy) and leech therapy (hirudotherapy) [6,7]. Diuretics were also known historically. Additionally, the foxglove plant (Digitalis) has been used as a remedy for various conditions, including HF; its usefulness as a therapy for congestive HF was first described by William Withering in the 18th century [8].

A major step in the understanding of the hemodynamics of HF was taken with the introduction of heart catheterization in the 1940s, when the concepts of forward and backward failure were introduced [9]. The main pharmacological treatments for HF in the middle of the 20th century were diuretics and digitalis, and the patients were recommended bed rest and restricted fluid intake [10]. More effective loop diuretics were introduced in the late 20th century (furosemide patented 1959).

As a treatment for severe HF refractory to pharmacological treatment, LVADs were introduced. The first implantation performed by Michael DeBakey in 1966 [11], and the next year the first heart transplantation was performed by Christiaan Barnard [12].

In the1980s, the concept of neurohormonal activation was introduced replacing previous theories of fluid overload as the cause of heart failure and pump failure. Thus, increased and persistent activation of the sympathetic system and RAAS was highlighted as a response to long-term reduction of arterial blood pressure and cardiac output in HF. Furthermore, it was put forward that these hemodynamically initially positive neurohormonal responses over time negatively affected long-term cardiac remodelling, symptoms and ultimately outcome. In support of this concept, large randomized studies eventually confirmed that treatment with antagonists to the renin-angiotensin and sympathetic nervous systems, i e ACEIs, ARBs, MRAs and BBs, reduced mortality and morbidity in HF [10,13]. In later years several new treatment options have been shown to further improve HF and outcome, namely ICD/ CRT, ARNi (acting to support increase of the neurohormonal system by specifically inhibiting degrading of natriuretic peptides), sinus node inhibitor (ivabradine) and exercise physiotherapy [13]. These treatments are effective in HFrEF but have to date not been proven for HFpEF.

2.2 DEFINITION

Historically different definitions of HF have been used using a combination of clinical signs and findings, e.g. the Framingham criteria [14], and later with the use of echocardiography and natriuretic peptides in European criteria [15] and others. These different definitions of HF show, however, considerable similarities [16].

The current guidelines from the European Society of Cardiology (ESC) define HF as a clinical syndrome, characterized by symptoms and/ or signs, typically dyspnoea, oedema and fatigue. This syndrome is caused by a cardiac abnormality, in most cases related to a dysfunction in the LV, leading to reduction of CO and/ or elevation of the intracardiac end-diastolic pressure, or maintaining CO through elevated filling pressures. These findings may be present already at rest or revealed under "stress" or physical exercise [13]. Notably, guidelines emphasize the importance of diagnosing the underlying cardiac abnormality and aetiology (see below paragraph 2.5) in all cases of HF.

2.2.1 Definition based on LV ejection fraction

In the current ESC guidelines HF is divided into three different groups depending on LVEF. Patients with LVEF ≥50% have HFpEF (previously named diastolic HF), while those with LVEF <40% have HFrEF (previously named systolic HF). The group with LVEF in the range of 40-49% is said to have HFmrEF – and is regarded to be a "grey zone", meaning that such patients may have some indications of HFpEF and HFrEF concomitantly [13].

HFpEF and HFmrEF are groups defined by LVEF as a normal or near normal criteria (EF) and thus other objective abnormal diagnostic criteria are needed to establish the diagnosis of LV HF with LVEF >40%, see below under paragraph 1.3.1. These other criteria are similar for HFmrEF and HFpEF in the present European guidelines (ESC 2016) and are described below in section 2.3.2 under the common denominator HFpEF.

2.2.2 Definition according to the temporal course of HF

The ESC guidelines [13] propose terms as "de novo, or acute onset HF" for patients whose symptoms emerge and develop rapidly. Possible causes of acute HF are ACS, uncontrolled HT, arrhythmia or pulmonary embolism. The term "chronic HF" is used for patients who have had the disease for some time, "stable HF" for HF patients whose symptoms have been unchanged for a month, and "decompensated HF" for stable HF patients whose disease deteriorates.

2.2.3 Definition according to the severity of HF symptoms

The ESC guidelines [13] recommend the use of a grading system of severity of chronic HF symptoms, i.e. the NYHA classification. Thus, NYHA Class I describes a HF patient with no limitation of physical activity, and NYHA class IV refers to HF patients who cannot carry on any physical activity without symptoms.

Another classification system is the Killip classification (with grades from Class I to Class IV). This classification is used to grade the severity of acute HF symptoms, often after a myocardial infarction [17].

NYHA and Killip classifications do not overlap as they are used during different circumstances, both classifications use the scale I-IV for increasing degree of severity, which is illustrated in Table 2.

NYHA class	Physical limitation and symptoms (NYHA)		Killip class	HF signs (Killip)
I	No limitation, no symptoms		I	No signs
II	Mild limitation, symptoms at significant exertion		II	Pulmonary crackles <10 cm, elevated JVP, third heart sound
III	Significant limitation, symptoms at mild exertion		III,	Pulmonary edema
IV	Symptoms at rest		IV	Cardiogenic chock

Table 2. Comparison of NYHA and Killip HF classifications.

2.3 DIAGNOSIS

The ESC guidelines [13] advocate an algorithm for diagnosing HF. This algorithm is based on evaluation of several aspects: the patient´s previous health history and etiological factors for HF, such as history of IHD, further presence of symptoms and signs of HF, and presence of any abnormality on ECG. If ≥ 1 abnormality is found on this evaluation, the work-up should continue with an echocardiogram to confirm or reject the HF diagnosis. When available, natriuretic peptides, such as BNP or NT-proBNP should be used early to help diagnose HF, irrespective of LVEF.

2.3.1 Diagnosis of HFrEF

The key feature of HFrEF is that LVEF is reduced <40% [13]. However, LVEF is only one of several methods to evaluate the systolic function of the LV, other echocardiographic methods being dP/dt or Global Longitudinal Strain or other methods/techniques [18,19]. Another method used to assess LV systolic function is left heart catheterization that allows measurement of cardiac output, although this invasive method is not recommended by the Guidelines for a routine heart examination [20,21]. The current ESC HF Guidelines also recommend cardiac magnetic resonance imaging as a gold standard for LVEF evaluation, and cardiopulmonary exercise test for evaluation of the cause of dyspnoea and as a part of a heart transplantation work-up [13].

2.3.2 Diagnosis of HFpEF

The key feature of HFpEF and HFmrEF is believed to be DD and/or structural disease of the LV. This diagnosis does not include HF conditions caused by valvular heart disease or isolated right ventricular failure [13]. An impaired or delayed early relaxation ability is the first finding in HFpEF. The early relaxation of the LV is an active, energy-demanding process, and therefore susceptible to disturbance in conditions with depletion of energy, e.g. myocardial ischemia [22]. This progresses on to impaired compliance in late diastole, when the left chamber is passively filled with blood and end-diastolic filling pressure will rise [23]. LV remodelling and progression into systolic dysfunction may develop, but this pathway has not been clearly proven [24]. Diastolic LV dysfunction may also progress without increase of LV size but into stiffening of the heart, and LVEF will be preserved or normal. The most frequently used grades of DD are mild, moderate or severe. This grading is clinically important as it is known that a deterioration of diastolic function leads to worsened heart failure and increased mortality [25,26].

During the past 10 years, there have been several different methods to diagnose HFpEF. In an early ESC Consensus Document [27] the following criteria are listed: a combination of symptoms or clinical signs of HF, and a non-dilated LV with LVEF >50%. Thereafter, the diagnostic work-up could take three different pathways, all with different methods to find elevated LV filling pressures: 1) right heart catheterization, 2) echocardiography and 3) measurement of natriuretic peptides. Some of the measures are complex in clinical praxis, like catheterization and measures of LV stiffness (i. e. the constant Tau).

A newer algorithm for diagnosing HFpEF is proposed in the present American and European echocardiography guidelines [28]. It is based on several echocardiographic variables: e´, average E/e´, LAVI. In addition, if TR is detectable, its flow velocity should be measured. These guidelines thus suggest the use of multiple echocardiographic variables (at least two) for better diagnostic accuracy. These guidelines suggest how to grade the DD based on the above-mentioned echocardiographic variables, also including mitral flow profile (E/A ratio) and the blood flow velocities in the pulmonary veins. Diastolic dysfunction is graded: I (mild), II (moderate) and III (severe).

Finally, the current ESC Guidelines [13] suggest the following criteria for HFpEF diagnosis: clinical signs and symptoms of HF, normal (preserved) LVEF on echocardiography and elevation of blood levels of natriuretic peptides. In addition, there must be at least one additional structural abnormality detected by echocardiography, such as increase of LVMI or LAVI, or additional signs of DD (elevation of E/e´ and decrease of e´). Other defined criteria may be used if these parameters are not available.

These guidelines also suggest how to grade and name DD: “impaired relaxation”, pseudo-normal filling” and “restrictive filling”, corresponding to the above-mentioned grades I, II and III, respectively.

The three different ways of assessing the biochemical and echocardiographic parameters are listed in Table 3 below, presenting normal values for these parameters (modified from Paper I).

In a recent European consensus recommendation, a more comprehensive algorithm for HFpEF work-up was presented. It includes several steps. Firstly, a pre-test assessment is to be made, comprising risk factors, ECG, standard echocardiography, measurement of natriuretic peptides and exercise testing. In the next step a comprehensive echocardiography is performed with measuring of various parameters for diagnosing HFpEF. These parameters are graded according to a scoring system. In case of a high score, the HFpEF diagnosis can be made. In case of an intermediate score, additional tests can be carried out, such as stress echocardiography or left and right cardiac catheterization. Finally, the aetiology of HFpEF should be assessed [29].

Variable	Consensus statement 2007	ESC Guidelines 2016	ASE/EACVI 2016 Guidelines
Biomarkers	BNP ≤200 pg/ml or NT-proBNP ≤220 pg/ml	BNP <35 pg/ml or NT-proBNP <125 pg/ml	not defined
LVEF	≥50%	>50%	≥50% ("grey area" between 40 and 49%)
e´	not defined	≥9 cm/s (average)	septal ≥7 or lateral ≥10
E/e´	≤15	<13	≤14
E/A	≥0,5	normal (or pseudo-normal) 1-2	normal (or pseudo-normal) 1-2
DT	≤280 ms	140-220 ms	≥150 ms
IVRT	not defined	60-110 ms	not defined
TR	not defined	Mentioned, but limits not defined.	<2.8 m/s
LVMI	♀ ≤122 g/m^2, ♂ ≤149 g/m^2	♀ ≤95 g/m^2, ♂ ≤115 g/m^2	not defined
LAVI	≤40 mL/m^2	≤34 mL/m^2	≤34 mL/m^2

Table 3. From: Persson, Matan et al. Int J Cardiol. 2019 Jan 1; 274:202-207.

2.4 EPIDEMIOLOGY

HF prevalence is increasing, especially in the developed countries [30]. The incidence of HF in the Western countries is estimated to 5-10 per 1000 person-years, and the prevalence is about 2% of the adults, but as high as 12% among the elderly (≥80 years old) [31,32]. In Stockholm County, Sweden, HF prevalence is similar 2%, and incidence 3.8/1000 person-years [33] which indicates around 7000 new-onset HF cases a year in Stockholm, with an estimated population of 2 million people.

HF often leads to admission for in-hospital treatment, with a high rate for re-admissions (ca 50% per six months) [34-36]. Thereby HF carries a high cost for the society [37]. Both the

costs for hospital care and the prevalence of HF are expected to rise considerably during the coming 20 years [32].But there are different views on the future evolution of HF prevalence. According to an estimation by American Heart Association [32], there will be a significant rise in the HF prevalence during the next 10-15 years, while a recent Swedish study [38] showed that an overall slight decrease in the HF prevalence is to be expected, although there seem to be an unexplained increase in younger patients, partly related to increased prevalence of obesity shown to be associated with HF [39].

About 40-50% of HF patients have HFpEF [40], and there is evidence that this share is increasing presumably due to the ageing population [32]. In addition the definition of HFpEF changes and may influence these figures.

2.5 AETIOLOGY/ PATHOPHYSIOLOGY

2.5.1 Aetiology

The current ESC guidelines [13] list three major disease groups as aetiology of HF:

1. myocardial disease, most commonly IHD, but also myocarditis, infiltrative diseases, alcohol- and other cardiomyopathies that today need to be assessed for specific or often hereditary disorders,
2. loading conditions abnormality, most commonly HT, but also valvular disease, constrictive pericarditis, high output conditions, ie sepsis, thyreotoxicosis.
3. arrhythmias, most commonly atrial tachy-arrhythmias such as AF [41].

In the developed world, the most common conditions causing HF are IHD and HT (accounting for > 50% and 39-59% of the cases, respectively) [42,43]. In many patients, these conditions exist concomitantly.

There is evidence from a large Swedish study that improved treatment of IHD in the recent years has led to decreased incidence of HF as a complication to myocardial infarction [44].

Compared to the HFrEF population, the HFpEF patients are of higher age, with a greater proportion of women. Furthermore, HFpEF patients have a higher prevalence of HT, AF, and diabetes, and a lower prevalence of IHD [40].

In the present work we are not considering HFpEF caused by other forms of cardiac disease, such as presence of significant valvular disease, right ventricular disease, myocarditis, or more specific forms of cardiomyopathies, like hypertrophic cardiomyopathy whereas hypertensive heart disease as a cause of HF is included.

2.5.2 Pathophysiology

HF is characterized by a decreased CO, leading to decreased perfusion of the tissues during exercise or if severe at rest. Several compensatory mechanisms are being activated when the heart tries to elevate mean arterial pressure and to increase the tissue perfusion. At early stages of HF, the Frank-Starling mechanism is important, where the heart reacts to increased preload with increased stretch of the cardiomyocytes leading to increased end-diastolic volume, in order to maintain the stroke volume.
Neurohormonal activation also takes place, including release of catecholamines via the sympathetic nervous system and activation of RAAS. This leads to fluid retention and to

increase in both peripheral vasoconstriction and in heart rate, thereby increasing mean arterial pressure (MAP) and the afterload for the heart; an increase of the arterial stiffness in HF patients may contribute to further increase of afterload. As a result, there is a mismatch between the increased afterload and the reduced contractile performance of the heart, leading to an increase of the end-diastolic venous pressure in the lungs (increased preload), and eventually, to a clinical picture of acute decompensated HF with pulmonary effusion and decreased peripheral perfusion [45,46].
The neurohormonal activation promotes cardiac remodelling with enlargement, hypertrophy and deterioration of LV function into HFrEF in many patients. With increased age, there is an increase in the stiffness of the LV, and therefore, a worsening of the DD [47].
Natriuretic peptides, such as BNP and atrial natriuretic peptides, are hormones released from the cardiomyocytes in the ventricles and the atria respectively as a response to the distension of the heart. These peptides are physiologically active, counteracting the vasoconstricting effect of the neurohormonal activation by causing vasodilation, promoting excretion of salt and water and counteracting secretion of renin and aldosterone.
Although effective in the early stages, with continuing HF the chronic activation of compensating mechanisms leads to a hemodynamically worsening situation, with decreasing MAP and CO, increased fluid retention, and LV remodelling [48,49].

2.5.3 Possible pathophysiological mechanisms leading to HFpEF

For HFpEF, microvascular inflammation in conjunction with co-morbidities are suggested as the major drivers of disease rather than neurohormonal activation [50].

Although the pathophysiology of HFpEF is still uncertain there is evidence that this disease is distinctly different from HFrEF. These two HF phenotypes are likely to present different structural changes in the heart, and different responses to pressure and volume changes in the LV, where HFpEF is characterized by higher LV filling pressures than HFrEF [51]. Of note, development of long-term changes of LV function in HFpEF are insufficiently studied, even though there is evidence of worsening DD over time [47].

The extent of neurohormonal activation seems to be lower in HFpEF and HFmrEF than in HFrEF, although an association between neurohormonal activation and cardiovascular mortality is seen in all three HF phenotypes [52,53].

Recently, a new paradigm for HFpEF was proposed [50], comprising the following elements in the development of HFpEF: high prevalence of various comorbidities, an inflammatory state - both systemically and in the endothelium of the coronary microvasculature, increased stiffness of cardiomyocytes due to low protein kinase G - activity with hypo-phosphorylation of the giant muscle protein titin [54]. In line with this concept, a recent study showed that microvascular dysfunction, presumably driven by the inflammatory state, is highly prevalent in a large sample of patients with HFpEF [55].

Another pathophysiological mechanism put forward in HF is myocardial fibrosis [56] which may be due to development of myofibroblasts, a type of cell derived from fibroblast, which undergo transition under the influence of various signalling molecules such as transforming growth factor β and fibroblast-growth factor. Activated myofibroblasts synthesize and secrete collagens which are deposited in the interstitial space causing increased stiffness of LV myocardium. Interestingly, a study with myocardial biopsies performed in patients with HFpEF [57] showed a significant increase in both collagen-dependent and titin-dependent stiffness, thus suggesting that both increased fibrosis and changes in titin homeostasis are parts of the HFpEF pathophysiology. Imbalance of collagen turnover, i. e. synthesis, cross-

linking and degradation of collagen, may act differently in HFrEF and HFpEF where HFpEF has been ascribed to be characterized by elevated collagen synthesis [58].

2.6 NOVEL BIOMARKERS IN HF

2.6.1 Biomarkers of myocardial fibrosis

As myocardial fibrosis has been shown to be a part of the natural history of HF [57,59], there have been attempts to measure fibrosis biomarkers in blood as a "liquid biopsy". Two circulating biomarkers of collagen metabolism that can be measured, PICP and CITP, have been shown to correlate with myocardial fibrosis and myocardial collagen degradation, respectively [60]. PICP and CITP have been used as biomarkers also for other conditions affecting collagen metabolism, e.g. bone diseases [61,62]. A ratio of CITP:MMP-1 has been used as biomarker for cardiac fibrosis. MMP-1 is an enzyme that is involved in the process of degrading the fibrils of collagen I and III in the myocardium [63] and CITP:MMP-1 ratio is inversely correlated to the extent of collagen-crosslinking (CCL) of collagen type I, and may be viewed upon as a biomarker of collagen fibre "quality" [64,65].

In a recently published study it was found that elevated levels of PICP and CITP are associated with mortality in HFrEF patients (both cardiovascular and all-cause mortality), and elevated levels of CITP were correlated with a higher NYHA class [66]. In particular, increased degradation of fibrosis seemed to be important for poor outcome. In contrast, it has been proposed that HFpEF is characterized by an increased collagen synthesis [58]. Synthesized collagen fibrils are covalently crosslinked, thereby building collagen fibres with increased insolubility, thickness and stiffness [59,64], and this aspect of collagen synthesis should be taken into account when assessing collagen as a player in fibrosis.

It has also been shown that in HFpEF there is a correlation between the severity of HF and the levels of fibrosis biomarkers. Of note, there are findings supporting that MRA treatment have favourable effects on fibrosis and DD in patients with HFpEF [67] and that some biomarkers may modify the results of the treatment [68].

The fibrosis biomarkers studied in this thesis are summarized in the Table 4 below [60,64].

There are also other proteins that possibly could act as fibrosis biomarkers, as they are associated with a presence of myocardial fibrosis, e.g. N-terminal propeptide of procollagen type III and Galectin 3 [60]. For example, the blood levels of Galectin-3 are associated to DD [69]. Another biomarker for myocardial fibrosis is a soluble form of suppression of tumorigenicity 2 (sST2), a protein that belongs to the interleukin family. sST2 is closely related to cardiac hypertrophy, remodelling and fibrosis. It has been proposed as a biomarker for cardiac fibrosis, and also for diagnosis and therapy follow-up in HF [70,71]. However, these three biomarkers were not studied in this thesis.

Name of the biomarker	*Biomarker*	*Biological process assessed*
CITP	Formed during degradation of collagen type I	Collagen degradation.
PICP	Formed during synthesis of collagen type I from procollagen.	Collagen synthesis.
CITP: MMP-1	Ratio between collagen degradation and enzyme of degradation (MMP-1)	Degree of myocardial CCL (quality of collagen fibres; low ratio - high fibre ”resistance”)

Table 4. Various biomarkers of cardiac fibrosis. Abbreviations in Table 4: CCL=collagen cross-linking, CITP=carboxy-terminal telopeptide of collagen type I, MMP-1=matrix metalloproteinase-1, PICP=carboxy-terminal propeptide of procollagen type I.

2.6.2 Extracellular vesicles

The current ESC HF Guidelines [13] recommend the use of blood biomarkers such as natriuretic peptides for diagnosis and as potential prognostic tools. There is, however, increasing interest in identifying new biomarkers which could be used in the discrimination of HF phenotype, in risk stratification and prognosis, for therapy guidance, and even to be used as potential drug targets. Indeed, there has been increasing focus on the potential usefulness of extracellular vesicles (EVs) as biomarkers for cardiovascular diseases, including HF [72]. EVs are small extracellular vesicles with a diameter between 100 and 1000 nm, which bud off from plasma membranes of various cells [73]. Under normal conditions, most of the circulating EVs originate from the platelets. Less than 10% originate from leukocytes and <5% from endothelial cells or other cell types. EVs are released into the circulation as a response to different physiological or pathophysiological stimuli (e.g. hypoxia, ischemic injury, or shear stress). They may exert a variety of biological functions by interacting with various cells in several different ways: e.g. by releasing “signal molecules”, or by fusing with cells through interactions with cell surface-located molecules [74].

Elevated blood levels of EVs, mainly derived from platelets, have been shown in various diseases, such as acute coronary syndrome [75,76], sepsis/endotoxemia [77], or acute ischemic stroke [78], following acute traumatic brain injury [79] but also following exercise, mental stress and during experimental inflammation [80-82]. Of note, EVs can be measured in other body fluids, e g cerebrospinal fluid, where elevated levels have been measured in amyotrophic lateral sclerosis [83] and schizophrenia [84].

EVs from vascular endothelial cells may be released in response to endothelial dysfunction, due to e.g. oxidative and shear stress, and have been reported to be associated with progression of HF in animal models [85]. One study suggests that the ratio between endothelial progenitor cells and endothelium-derived EVs can be used as a biomarker for chronic HF and for better discrimination between HFpEF and HFrEF [86].

Although there has been considerable research on EVs in various diseases including cardiovascular disease, few studies have been performed on EVs released from the human heart [72]. Furthermore, the research on EVs in HF is not extensive. Therefore, the present thesis focused on the exploration of EVs of potential cardiomyocyte origin and its possible relationship to HF.

2.7 PROGNOSIS

HF is a condition associated with a high mortality [87]. A recent European study found an all-cause mortality at 17% and all-cause hospitalization rate at 44% in patient with acute HF one year after diagnosis. For patients with stable chronic HF the numbers were 7% and 32%, respectively [88]. In Sweden, the results from Swedish HF registry showed 1- and 5-years mortality of 18.8% and 54.5%, respectively [89]. Both morbidity and mortality in HFrEF are reduced by HF treatment. Of note, HFpEF has almost as poor prognosis as HFrEF [23,90], but besides treatment of symptoms and various comorbidities there is presently no evidence-based treatment that improves prognosis in HFpEF.

2.8 TREATMENT

2.8.1 Pharmacological treatment of HFrEF

The current ESC guidelines suggest the following drugs as cornerstones in the therapy: ACEIs or ARBs (the latter to be used especially when ACEIs are not tolerated or are contraindicated) and BB. Besides that, in all symptomatic patients with LVEF $\leq$ 35%, a mineralocorticoid receptor antagonist (MRA) should be added [13]. These recommendations are based on the results of large randomized controlled trials (RCT) performed in patients with HF and reduced LVEF. For instance, the CONSENSUS and the SOLVD-Treatment trials showed a significantly reduced mortality in HF patients treated with ACEI, and the SOLVD-Treatment trial demonstrated a reduced rate of HF-related hospitalization [91-93]. Also in HFrEF patients without overt symptoms of HF decreased mortality could be achieved by treatment with ACEI [94,95].

Regarding ARB, two trials have shown a significant reduction in HF-related hospitalization: VALHEFT and CHARM-Added. In addition, the CHARM-Added trial showed a reduced risk of cardiovascular death [96,97].
Of note, in a post-hoc analysis of the CHARM study population with HFmrEF (LVEF 40-49%) it was seen that candesartan improved the combined outcome of cardiovascular death and HF hospitalisation in patients with HFmrEF as well as in those with HFrEF [98].

Concerning BBs, there are three large RCT: CIBIS-II, COPERNICUS and MERIT-HF; all of them showed a significantly reduced mortality and HF-related hospitalization [99-101].

The RALES trial, which investigated the effect of the MRA spironolactone vs placebo, showed a significant reduction in both mortality and in HF-related hospitalization. Another

large trial, the EMPHASIS-HF which studied eplerenone vs placebo, showed that both cardiovascular death and HF-related hospitalization were significantly reduced irrespective of HF aetiology and with NYHA II functional class [102,103].

The guidelines also recommend diuretics in HF patients with symptoms of congestion, especially in cases of acute decompensated HF [104,105]. Any disease-modifying effect of the diuretics have not been shown in RCTs, but in a systematic Cochrane review of few small trials there was some evidence that they may reduce mortality and morbidity, and improve the exercise capacity [106].

In addition, the angiotensin receptor neprilysin inhibitor (ARNI, valsartan/sacubitril), should be used instead of ACEI or ARB in patients with LVEF ≤35% who remain symptomatic despite of optimal treatment (including ACEI or ARB). These recommendations are based on the recently performed PARADIGM-HF trial which showed that in this patient population ARNI reduced both HF hospitalization rate and mortality (overall and cardiovascular) more efficiently than ACEI [107].

An I_f-channel inhibitor (ivabradine) is recommended by the guidelines in patients with LVEF ≤35% with sinus rhythm and with heart rate ≥70 beats per minute despite OMT including BB in maximal tolerated dose. Ivabradine inhibits the I_f channel in the sinus node and thereby reduces the heart rate, and in an RCT performed a few years ago (the SHIFT study) it was shown to reduce both the HF-related mortality and hospital admissions in HF patients [108].

During the last 3 years, treatment with sodium-glucose cotransporter-2 (SGLT-2) inhibitors was shown to improve prognosis for HFrEF and HFmrEF patients, also those without a concomitant diabetes diagnosis [109,110]. One possible mechanism of the SGLT-2 inhibitors is reduction of fibrosis and inflammation in the myocardium [111]. However, such treatment has not yet been recommended by the ESC HF Guidelines.
HFrEF patients with iron deficiency are currently treated with ferric carboxymaltose (FCM) administered intravenously as symptom alleviation [13]. Recently, a new randomised, double-blind and placebo-controlled trial was completed (AFFIRM-AHF), showing that treatment with FCM given to HFrEF and HFmrEF patients presenting with acute HF and iron deficiency led a near-significant (p=0.059) reduction of mortality and HF-related hospitalizations [112].
Very recently, a new drug was presented: omecamtiv mecarbil, which is a selective activator of cardiac myosin that increases the contractile force of the myocardium. This drug is evaluated in the ongoing GALACTIC-HF, a placebo-controlled randomized trial with a composite outcome of the first HF event or cardiovascular death; the results are expected to be presented in 2021 [113].
Another promising class of drugs for HFrEF treatment are soluble guanylate cyclase stimulators (sGCs), acting by mediating formation of cyclic guanosine monophosphate that in turn is a mediator for vasorelaxation and reduction of cardiac hypertrophy and fibrosis. Currently, a phase 3-trial is ongoing for a sGC called vericiguat [111].

2.8.2 Pharmacological treatment of HFpEF/ HFmrEF

Importantly, there is no established treatment for HFpEF [114], and according to the current ESC guidelines [13], the same applies to HFmrREF, as both diagnoses have been included in the same treatment trials.

The guidelines suggest symptomatic treatment of congestion with diuretics, regulation of blood pressure in patients with HT, and oral anticoagulant (OAC)-treatment in patients with AF. There is some/minor evidence of symptom alleviation with ACEI-treatment [115] and reduction of HF-related hospital admissions with ARB-treatment [116] but mortality benefits

have not been shown. Two sub-studies of I-PRESERVE and TOPCAT suggest that MRAs or ARBs may improve outcome in low risk HFpEF patients (low levels of natriuretic peptides) in contrast to patients with high risk HFpEF (high levels of natriuretic peptides) [117,118].

Recently, a large multi-center RCT (PARAGON-HF) comprising 4822 patients has been completed, showing that ARNI were not superior to ARB for preventing cardiovascular death and HF hospitalization in patients with HFpEF or HFmrEF [119,120]. The reduction of HF-related hospitalization was larger in women than in men, which, however, is difficult to interpret clinically as these results were found in a sub-group analysis of the PARAGON-HF trial [121].

A registry-based RCT (SPIRRIT-HFpEF) is currently ongoing in Sweden, aiming to enroll 3500 patients and with the hypothesis that MRA (Spironolactone) will be effective in reducing mortality and morbidity in patients with HFpEF [122].

2.8.3 Non-pharmacological treatment

The current ESC guidelines recommend regular aerobic physical exercise in HF patients regardless of LVEF as it has been shown to reduce the risk of hospital admission and to increase the quality of life [123]; in HFpEF physical exercise has also shown to lead to increased peak oxygen consumption and improved diastolic function of the LV [13].

In HFrEF patients with LVEF ≤35% who in spite of OMT remain symptomatic with NYHA Class II–III, treatment with implantable cardioverter defibrillator (ICD) is recommended to reduce sudden cardiac death. ICD treatment has been shown to reduce overall mortality in this patient category significantly - by 23% in one study [124]. A large meta-analysis comprising eight trials and >5000 patients showed a significant reduction of both arrhythmia-related and all-cause mortality (relative risk 0.40 and 0.73, respectively) [125].

For the same category of patients, treatment with disease modifying cardiac resynchronization therapy (CRT) is recommended if the ECG shows sign of ventricular dyssynchrony. In the CARE-HF trial a significant reduction of both HF-related and sudden death due to the CRT treatment was observed [126]. A large meta-analysis comprising five randomized controlled trials with >5000 patients showed that both mortality and morbidity were significantly reduced by CRT treatment [127]. Sub-studies of REVERSE and MADIT-CRT studies indicate CRT benefits in patients with higher LVEFs than 30-35% suggesting that in the presence of LBBB the correction of electrical dyssynchrony by CRT may be beneficial across a wider range of low LVEFs [128]. There is some evidence that also HFrEF patients with AF can benefit from CRT treatment, although AF patients are older and have a poorer prognosis than patients without AF [129].
In HFpEF, there is no indication for ICD (unless for secondary prevention of sudden cardiac death) or CRT [130]. Generally, ICD and CRT therapy is under-used in Sweden [131].
For patients with end-stage HF, i.e. patients who are severely symptomatic (NYHA class IV, and NYHA class III in some selected cases) despite OMT, the guidelines recommend considering treatment with LV assist device (LVAD), either as a long-term therapy, or as a temporary therapy (during the time on the waiting list) in patients who are eligible for heart transplantation which is still the golden standard therapy in end-stage HF, with a median postoperative survival of nearly 11 years [132]. However, heart transplantation is a therapy that never has been evaluated in any randomized controlled studies.

2.8.4 Organization of HF care

In general, including in Sweden, HF patients treated in the primary and hospital care often do not receive guidelines indicated HF medication [133-135]. As an example, improved adherence to the clinical guidelines regarding indication for OAC in a restricted geographic area (e. g. a county) resulted in better clinical outcome, with decrease in incidence of ischemic stroke [136,137].

Both international and national guidelines emphasize the importance and evidence of multidisciplinary teams (MDTs) in diagnosing and following-up of HF [13,135,138]. A physician and a nurse, both specialized in HF, should be parts of such a team, among other health care professionals. The HF MDT should perform the following tasks: diagnosing HF, patient education, starting and adjusting HF medication, and continuing HF management in patients with end-stage disease. There is evidence that HF management carried out by MDTs reduces both mortality and hospital admissions in HF patients [139].

A large meta-analysis comprising 48 different studies showed that better compliance to drug treatment for HF led to a significantly reduced risk for both hospitalization and death; several different strategies for improving compliance have been described: patient education, structured follow-up routines, patient web-portals etc. [140]. An international registry study including >6000 patients with HFrEF showed that their clinical outcome (both all-cause mortality and HF-related hospitalization) was improved if their physicians had a better adherence to the treatment guidelines [141].

Another meta-analysis comprising 29 different studies [142] could identify several strategies to reduce HF-related hospitalizations, e.g. patient education, telephone follow-up, tele-monitoring etc. Of these methods two were recognized as the most efficacious, reducing also mortality and all-cause hospitalizations in HF-patients: follow-up by a specially trained staff (e.g. HF nurses) and access to special HF clinics. Yet HF clinics and MDTs have not been introduced on a larger scale in Sweden or other countries [143].

3 AIMS

3.1 PAPER I

The main aim of this study was to evaluate whether patients with acute symptoms and signs of HF and preserved LVEF met the criteria for HFpEF diagnosis according to the current ESC guidelines [13]. To verify the probable HFpEF diagnosis the patients were examined with echocardiography 4-8 weeks after enrolment in the study, whereby various diagnostic echocardiographic parameters were recorded. Another aim of the study was to evaluate whether these echocardiographic parameters affected the patient outcome of the study, which was death of all causes or HF-related hospitalization.

3.2 PAPER II

HF patients in Stockholm were previously treated in primary care after diagnosis and acute handling during hospital admissions and their long-term treatment is often not in line with the requirements in the international guidelines [133]. There are few specialized HF nurses employed in primary care, and hospital-based HF clinics are not appropriately sized, creating an unmet need [143]. This can lead to delayed and insufficient treatment of HF [144].

The 4D HF Project was started in 2012 by Stockholm County Council and Karolinska Institutet with the aim to improve HF management in the Stockholm County, by optimizing

the structure of HF care and facilitating the implementation of evidence-based HF therapy in a wide multidisciplinary collaboration project. This clinical improvement program is separate from a research project that also was performed and where studies presented in this thesis are parts of this HF research project.

3.3 PAPER III

In this study, we investigated EVs in blood samples collected from coronary sinus, radial artery and right atrium during CABG. The EVs were phenotyped using different antigens of cardiomyocyte origin (Connexin-43, Caveolin-3, Troponin T and N-Cadherin), and antigens reflecting endothelial dysfunction (VE-Cadherin) and inflammation (PTX3 and MPO), i.e. pathophysiological mechanisms of relevance in HF but especially in HFpEF.
The aim was to investigate whether EVs were generated in the heart as tested by trans-coronary concentration gradients, and to study them in the two HF phenotypes HFrEF and HFpEF. Of note, the HF phenotypes were proxy diagnoses of HFrEF and HFpEF and compared with patients with normal LV function as previously presented in another study performed by our group [145].

3.4 PAPER IV

Our hypothesis was that, in our unique new-onset HF population, there would be significant differences between the two types of HF (HFpEF and HFrEF) in circulating fibrosis biomarkers. We hypothesized that patients with new onset HFpEF would have higher levels of PICP and lower values of CITP:MMP-1 compared to patients with new onset HFrEF. We also expected that patients with HFrEF would have higher levels of CITP based on findings in chronic HF [66,146].

Another aim was to further explore the role of inflammation in the HFpEF pathogenesis by measuring circulating VCAM-1. This molecule mediates adhesion of leukocytes to the vascular endothelium and plays an important part in activation of leukocytes during inflammation. Further, VCAM-1 has shown a correlation with risk of development of HF in patients who have had a myocardial infarction [147].

4 METHODS

4.1 STUDY METHODOLOGY

4.1.1 Paper I

4.1.1.1 Patient inclusion

This sub-study was a part of the Karolinska-Rennes (KaRen) study, the description of which can be found elsewhere [148]. KaRen was a prospective multicentre cohort study in which clinical and echocardiographic parameters in HFpEF and their impact for prognosis were evaluated [149]. Patient inclusion was performed between 2007 and 2011 in hospitals in France and Sweden. Patients who were included in the study had a clinical picture of acute HF according to the Framingham criteria [14], preserved LVEF (≥45%) and slightly increased levels of BNP (>100 ng/L) or NT-proBNP (≥300 ng/L).

4.1.1.2 Follow-up with a core lab echocardiography

The patients had a follow-up visit after 4-8 weeks when an echocardiography was performed. It was done in accordance with a checklist, and for all the examinations the same type of equipment was used. Eight different echocardiographic criteria were analysed in this study in accordance with the ESC guidelines [13]. Two criteria were used for assessment of structural abnormalities of the heart: LAVI and LVMI. Six criteria were used for assessment of the diastolic function: IVRT, DT, E/A, average e´, E/e´ and peak flow velocity of TR.

4.1.1.3 Grading of the diastolic LV function

In this study, we performed a grading of the diastolic LV function, based on the cut-off values presented in the ESC guidelines and consensus documents [13,27] and also described in the recommendations from EACVI and in previous papers in the KaRen study [28,148]. According to these considerations, 6 echocardiographic parameters were chosen out of a total of 8 different parameters available. A study with similar methodology has been published by another group [150]. Following grades for DD were used: 0 (none/ normal function), 1 (mild/ abnormal relaxation), 2 (moderate/ pseudo-normalisation), 3 (severe/ restrictive filling pattern). The grading system is summarized in Table 5.

Grade of DD	E/A	IVRT	DT	E/e'	TR	LAVI
Grade 1	<0.5, or					
		≥110 ms, or				
				<13 and		≥34 mL/m^2, or
					<2.8 m/s and	≥34 mL/m^2
Grade 2	0.5-2 and			≥13, or		
		55-110 ms and		≥13, or		
			150-280 ms and	≥13, or		
	0.5-2 and				≥2.8 m/s, or	
		55-110 ms and			≥2.8 m/s, or	
			150-280 ms and		≥2.8 m/s	
Grade 3	>2 and			≥13, or		
		<55 ms and		≥13, or		
			<150 ms and	≥13, or		
	>2 and				≥2.8 m/s, or	
		<55 ms and			≥2.8 m/s, or	
			<150 ms and		≥2.8 m/s	

Table 5. Grading of DD by echocardiographic parameters. From: Persson, Matan et al. Int J Cardiol. 2019 Jan 1; 274:202-207.

The follow-up was performed by calling up the patients on telephone and reviewing the death registry charts. This was done until a primary event or once every half a year until 18 months after the enrolment in the study was closed. Thus, for each patient without a primary event there was a follow-up time of 18 months or more. The primary endpoint of this study was time to all-cause death or to the first HF-related hospitalisation.

4.1.2 Paper II

4.1.2.1 Classification of patients and outcomes

The patient characteristics were extracted from VAL (Vårdanalysdatabasen), which is the database for health care analysis in the Stockholm County. A presence of 10 different comorbidities was examined using the ICD-10 codes that could be found during 10 years prior to inclusion. If a diagnosis of HF was present at any time during 5 years prior to inclusion, the patient was regarded to have HF. The Swedish national register for prescription of medication (Läkemedelsförteckningen) was used as a source for information on dispensation of the prescribed HF medicines, both so-called basic treatment (RAASi and BBs), and extended treatment (basic treatment combined with MRAs).
Health care administrative systems were used for extraction of data such as diagnosis, history of use of HF medication and HF-related hospital admissions. All patients who were discharged from inpatient care in one of the five emergency hospitals in the Stockholm County between 2012 and 2017 with HF as main or second position diagnosis were followed during one year after the discharge. The number of visits to the five HF outpatient clinics was also monitored.

4.1.2.2 Improvement of HF care

The project comprised several steps. A common work-up procedure for HF including an echocardiography protocol was developed, to be used by all caregivers in the Stockholm County who handle HF patients (Paper II, Supplementary material). It was done during years 2012-2013 by working groups including all levels of caregivers, dedicated to different goals in HF management, e.g. diagnostics, treatment or educational efforts. Comprehensive educational efforts were carried out towards the primary care with the intention to increase the awareness of HF. A management group of the project could then identify following problems: 1) too few of the HF patients were treated at specialized HF outpatient clinics, and 2) there was a need of additional financial resources to these clinics so a substantial improvement of HF treatment could be achieved, hopefully leading to a better outcome for the patients. Additional financial resources were allocated to the existing five hospital-based HF outpatient clinics during years 2014 - 2017, allowing each one of them to employ an extra HF nurse and a cardiologist. A decision was made by the steering committee to allocate resources to the existing outpatient HF clinics at the emergency hospitals, rather than to build up new outpatient clinics in the primary care.

Between 2012 and 2017 the following prospectively chosen parameters were registered in this study regarding the population in the Stockholm County: 1) referrals and visits to the five HF outpatient clinics, 2) numbers of patients who were admitted with HF diagnosis at the seven emergency hospitals (adjusted per million inhabitants), 3) prescription of important guideline based HF medications dispensed after admission, and 4) one-year all-cause mortality or HF readmission per year 2012 to 2017 for hospital admitted patients.

The timeline of the project is illustrated in Figure 2.

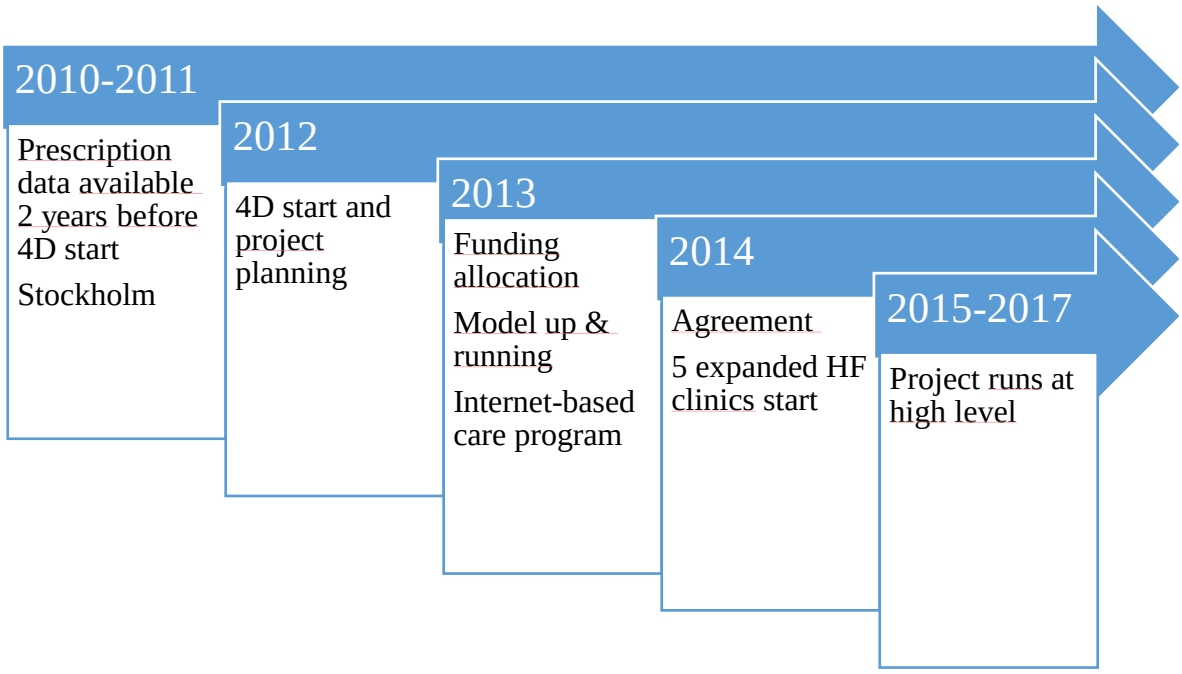

Figure 2. Timeline of the 4D project. From: Matan et al. Scand. Cardiovasc. J. 2020 (Supplementary material).

4.1.3 Paper III

4.1.3.1 Various EVs analysed

Blood samples were analysed for presence of EVs co-exposing myocyte-specific antigens Connexin-43 *and* Caveolin-3, and Connexin-43 *and* TnT. In the following we will use the following nomenclature: Connexin43/Caveolin-3 and Connexin43/TnT.
Analyses were also made for EVs exposing VE-Cadherin, MPO, PTX3 and N-Cadherin.
Flow cytometry was used to measure the EVs (see below for details).

4.1.3.2 Description of the EVs

EV phenotyping was thus performed through detection of different antigens (molecules) exposed on the EVs; monoclonal antibodies were used to detect the antigens (see below). We studied Connexin-43 which is a surface protein, expressed on different cells including cardiomyocytes. It is a gap-junction molecule and constitutes an inter-cellular connection which enables cross-talk between the cells. Of interest in the context of HF, lowered blood levels of Connexin-43 have been linked to worsening of congestive HF, both in animals and in humans [151,152]. We specifically investigated EVs that co-exposed Connexin-43 and Caveolin-3. The latter is a protein located in caveolae, (i.e., invaginations of the cell membrane) of myocytes in the heart and skeletal muscle. The function of Caveolin-3 includes cell membrane repair [153], vesicular transport and regulation of different cell-bound enzymes and receptors [154-156]. EVs exposing Connexin-43 and Troponin T (TnT) were also investigated. Troponin is a regulatory protein present in cardiomyocytes and involved in both contraction of the cardiac muscle, and an early phase of its relaxation in diastole [157]. It

is todays “standard biomarker” for myocardial injury but can also be used as a prognostic marker in HF [158,159]. Thus, two EV phenotypes which expose proteins of cardiomyocyte origin were assayed, to investigate a possible ongoing heart specific HF pathophysiology, and which we hypothesized could differ between HFpEF and HFrEF.

As HFpEF pathophysiology also may include endothelial dysfunction and inflammation [50], we investigated EV phenotypes that would represent these types of pathophysiology. We therefore assayed EVs exposing VE-Cadherin (vascular endothelial Cadherin) which is a protein that is expressed in the cells of vascular endothelium [160]. There is a significant correlation between the circulating levels of VE-Cadherin and endothelial dysfunction [80], which suggests that VE-Cadherin may reflect vascular disease. It is involved in regulation of vascular permeability and also in the reparative process of myocardial tissues after an ACS by stimulating angiogenesis [85,161].
EVs representing inflammation were considered to expose MPO or PTX3. MPO is mainly produced in neutrophil granulocytes and released into circulation when these cells are activated as a part of an inflammatory response [162]. The possible role of MPO in cardiovascular disease has recently been reviewed [163]. Interestingly, plasma levels of soluble MPO are elevated in HFpEF and in HFrEF, which could be explained by an inflammatory component of importance in the pathophysiology of HF, and relationships between MPO-levels and NYHA class suggest that MPO may be linked to severity of HF [164,165]. Regarding EVs exposing MPO, they have recently been put forward as novel biomarkers to detect renal involvement in ANCA-vasculitis [166]. Altogether, the literature on MPO makes it a molecule of interest in the context of HF.
PTX3 is “a relative” to CRP, but in contrast to CRP which is produced exclusively in the liver, PTX3 is produced locally by different cells, such as endothelial cells, leukocytes, and fibroblasts, in response to inflammation [167]. Plasma levels of PTX3 are elevated in HF, and PTX3 has been proposed as a prognostic marker in HF [168,169]. Being a locally produced inflammatory molecule, it is of interest to study its possible generation in the heart of patients with HF, and to explore possible differences between HFpEF and HFrEF.
N-Cadherin is like VE-Cadherin a junction molecule, but is mainly of myocardial origin and attached to myofibrils of the cardiomyocyte [170]. We hypothesized that it could be of interest to study this molecule in HF.

4.1.3.3 Classification of the patients

Before elective CABG the patients were classified by so called proxy diagnoses of HF, based on the results of the pre-operative work-up with echocardiography and blood analyses. The patients were divided into three groups with respect to presence or absence of proxy diagnosis of HF: HFrEF, HFpEF and Normal.
Patients with LVEF <45% were placed in the HFrEF group. Patients with LVEF ≥45% and echocardiographic findings diagnostic of diastolic LV dysfunction and/ or with elevated levels of natriuretic peptides were placed in the HFpEF group. Patient with no signs of LV dysfunction were placed in the Normal group. The diagnostical process is described more in detail elsewhere [145].

4.1.3.4 Blood sampling

During the surgical procedure before sternotomy new blood samples were taken from a radial artery and from the right atrium. Immediately after sternotomy, but before start of cardiopulmonary bypass, blood samples were taken from coronary sinus. All blood samples were frozen and stored at a temperature of -70 degrees Celsius.

4.1.3.5 Flow cytometric analysis of EVs

Samples were thawed in a water bath at 37 C° for 5 minutes and transferred into new tubes, then centrifuged at 2 000 g (gravitational force equivalent) for 20 minutes at room temperature. The upper supernatant was again transferred to new tubes and centrifuged at 20 800 g for 45 minutes at room temperature. The supernatant obtained by this second centrifugation step was discarded, and the EV-enriched pellet was used for the flow cytometric analysis. After that, 20 µL of the pellet was incubated for 20 min in dark, with 5 µl conjugated antibodies: anti-Connexin-43 Dylight 488, Dylight 633, Troponin T Dylight 755, VE-Cadherin Dylight 633, myeloperoxidase (MPO) Dylight 488, and Pentraxin 3 Dylight 755 (all analyses: Abcam, Cambridge, UK).

EVs were measured by flow cytometry on a Beckman Gallios instrument (Beckman coulter, Brea, CA, USA) with the threshold set to forward scatter. The EV gate was determined using Megamix-Plus FSC beads (0.3, 0.5 and 0.9 µm in size; BioCytex, Marseille, France). EVs were defined as vesicles less than 0.9 µm in diameter (forward scatter) and positive for antibodies described above. Conjugate isotype-matched immunoglobulin with no reactivity against human antigens was used as a negative control to define the background noise of the cytometric analysis. Results are presented as EVs/µL plasma, processed from the 20 µL pellet obtained after high-speed centrifugation.

4.1.4 Paper IV

4.1.4.1 Patient inclusion

Patients who were diagnosed with new-onset HF were included in the study as per the Stockholm PREFERS study protocol [2]. The inclusion took place either at inpatient clinics after the patients were admitted to hospital or at outpatient HF clinics.

4.1.4.2 Laboratory methods

For measurement of serum-PICP the EIA MicroVue CICP was used (Quidel Corporation, San Diego, Ca, USA). The inter-assay and intra-assay coefficients of variation were 12.0% and 8.1%, respectively, and the lower limit of detection was 0.2 ng/mL.
For measurement of serum-CITP a radioimmunoassay was used (Orion Diagnostica, Espoo, Finland). The inter-assay and intra-assay coefficients of variation were 10.0% and 9.9%, respectively, and the lower limit of detection was 0.6 ng/mL
For measurement of serum-MMP-1 an AlphaLISA was used (Perkin Elmer, Waltham, Ma, USA). The inter-assay and intra-assay coefficients of variation were 12.5% and 4%, respectively, and the lower limit of detection was 0.5 ng/mL. CITP and MMP-1 values were expressed in g/L and their ratio was calculated in each patient as previously reported [59].
For measurement of serum-VCAM-1 an AlphaLISA was used (Perkin Elmer, Waltham, Ma, USA). The inter-assay and intra-assay coefficients of variation were 13.2% and 16.8%, respectively, and the lower limit of detection was 3.1 pg/mL.

4.2 STUDY POPULATION

4.2.1 Paper I

There were 539 consecutive patients who were included in the KaRen study. After a follow-up visit 4-8 weeks later there were 356 patients left who had underwent an echocardiography that was analysed at a core lab.

The mean age of the patient was 76 years, and 57% of them were women. There was a high prevalence of various comorbidities, such as HT (79%), renal failure (44%), AF (37%), DM (32%).

4.2.2 Paper II

According to the Swedish national statistics authority, the population of the Stockholm County was 2 127 006 persons in 2012 and increased by 8.5% towards 2017. The number of admitted HF patients included in the follow up was 6284 in 2012, and 5794 in 2017 (a reduction by 8.5 % over the time period of five years). The mean age of the patients was 79 years, and 46% of them were women. The patients had high prevalence of various comorbidities, such as AF (62%), HT (74%) and DM (32%).

4.2.3 Paper III

This study is a part of CABG PREFERS study performed in Stockholm between 2015 and 2019, with all consecutive patients planned to undergo an elective CABG invited for inclusion [2]. A total number of 102 patients from CABG PREFERS were enrolled in this sub-study, but for some patients the blood samples were missing. Finally, there were 81 patients with complete blood samples from radial artery and coronary sinus, and 80 patients with complete blood samples from all three locations (radial artery, coronary sinus and right atrium). This is illustrated in Figure 1.
The mean age of the patients was 71 years, and most of them (90%) were men. The percentages of patients with HT was high (78%). A large percentage of the patients (32%) were in the NYHA class I. 28% of the patients had DM, predominantly type 2, and 12% have had previous myocardial infarction. 17% of the patients had a history of AF.

4.2.4 Paper IV

This study was a part of the PREFERS (Preserved and Reduced Ejection Fraction Epidemiological Regional Study) Stockholm, as illustrated in Figure 1. The design paper of the PREFERS Stockholm study includes an interim analysis of PICP and CITP after inclusion of at least 200 patients [2].

247 patients were enrolled in the study. Most of them (69%) had HFrEF. The mean age was 69 years, and almost three quarters were men (73%). The patients had a high prevalence of various comorbidities: 86% had HT, 62% had a history of AF, and 28% had DM. A high percentage of the patients (between 25 and 87%) were treated with HF medications. Compared to the HFrEF group the patients in the HFpEF group were older and were more often female and had higher prevalence of comorbidities, such as AF, HT and DM.

4.3 STATISTICS

In all four papers: continuous variables were presented with mean values ± SD if normally distributed, and otherwise with median and IQR. For presentation of categorical variables number of cases (n) and percentages were used.

4.3.1 Paper I

The data were split according to the definitions of cut-offs above. Univariate and multivariate Cox regressions were used for assessment of the prognosis. In the univariate analysis assessments were made whether the number of abnormal echocardiographic parameters had

an impact on the outcome, and in the multivariate analysis adjustments were made for age, gender, LVEF and natriuretic peptides.
Because some of the data was missing multiple imputations were used to impute missing values for continuous variables. After analyses of 25 complete data sets the results were used to generate valid statistical inferences. HR with 95% CI were used for assessment of association with the combined primary endpoint.
SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

4.3.2 Paper II

Cochran-Armitage test and multivariate Cox regression were used for evaluation of trends and outcome/ HR, respectively. As dependent variables in the regression analyses following parameters were used: time (year), comorbidities, gender, age (year) and OAC treatment. P-values <0.05 were considered significant.
SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

4.3.3 Paper III

The following statistical tests were used: Mann Whitney test for comparison of two groups of independent variables, Kruskal Wallis test for comparison of more than two groups of independent variables and Wilcoxon signed rank test for comparison of two groups of dependent variables. Correlations of non-parametric variables were studied with Spearman's rank correlation coefficient. When presented graphically, non-normally distributed categorical variables were shown with minimum, maximum and median values, and the IQR. P-values <0.05 were considered significant. IBM SPSS Statistics Version 25 program was used (IBM Corporation, Armonk, NY, USA).

4.3.4 Paper IV

For comparison of two sets of categorical variables Pearson Chi^2 –test was used, and for comparison of two groups of independent continuous variables Student's *t*-test for equality of means (two-tailed) was used. Pearson correlation coefficients were used for study of correlation between two groups of independent continuous variables. For exploring the explanatory value of the fibrosis biomarkers for development of HFpEF vs HFrEF univariate and multivariate logistic regressions were used. When presented graphically, non-normally distributed categorical variables were shown with minimum, maximum and median values and IQR. P-value <0.05 was considered significant. IBM SPSS Statistics version 25 program was used (IBM Corporation, Armonk, NY, USA).

4.4 ETHICAL CONSIDERATION

All the studies were performed in accordance with the Declaration of Helsinki.
In the studies described in Papers I, III and IV oral and written informed consent were obtained, and the studies were approved by the local Ethics Committee in Stockholm. For the Karen study described in the Paper I, an ethical permit was also obtained from CNIL (Comité National Informatique et Libertés), which is a French authority.
For the 4D HF Project described in Paper II ethical permit was not sought because this project did not start as a scientific study, but as a quality improvement project, and there were no plans to perform research within the program or to publish the results. However, the results were prospectively analysed for feedback to the caregivers as a quality evaluation of program implementation. A decision was then made to publish the project because of the results and its potential importance for the medical society and for the patients suffering from HF.

5 RESULTS

5.1 PAPER I

5.1.1 Patients

The mean time for follow-up in the KaRen study was 28 months, and 156 patients (43.8%) reached the combined primary endpoint [149].

Echocardiographic examinations showed that one ESC criterion for HFpEF diagnosis of LV structural heart disease was found in 92% (n=328) and one criterion of LV diastolic dysfunction in 82% (n=290). At least one criterion of these was found in 98% (n=351) and 94% had at least two criteria (n=333).

Secondly, diastolic LV function was graded according to the above-mentioned method (Table 3). 30% (n=107) of the 356 patients had mild DD, 27% (n=97) had moderate DD and 35% (n=124) had severe DD. Seven % (n=24) of the patients had normal diastolic LV function, while 1% (n=4) were not possible to classify because the data was difficult to interpret. In summary, the grading model used in this study made it possible to assess LV diastolic function in 99% (n=352) of the 356 patients that were included, and 92% (n=328) were abnormal.

5.1.2 Outcome

Cox multivariate regression analyses with adjustment for age, gender, LVEF and levels of natriuretic peptides showed two independent predictors of worse prognosis:
1) diagnosis of moderate and severe versus normal and mild diastolic dysfunction according to the above-mentioned model (Table 3), HR 1.8 (CI 1.2–2.7, p=0.0037).
2) presence of ≥4 out of 8 versus 0-3 abnormal diastolic echocardiographic variables, HR 2.0 (CI 1.3-3.1, p=0.0033). This is illustrated in Figure 3 (with an unadjusted value for HR presented).

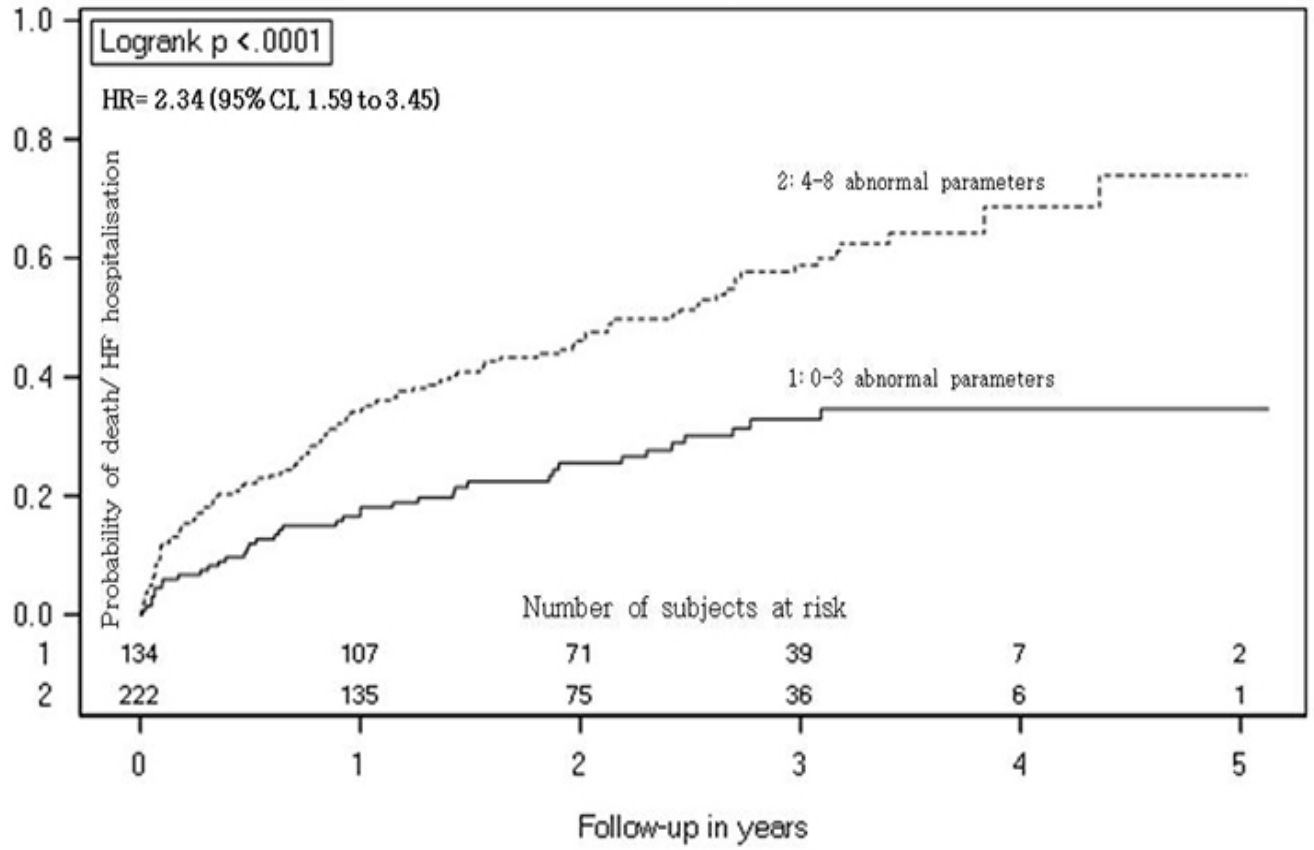

Figure 3. Number of abnormal echocardiographic parameters, and their impact on the outcome. From: Persson, Matan et al. Int J Cardiol. 2019 Jan 1; 274:202-207.

5.2 PAPER II

5.2.1 Patients

As mentioned above (chapter 4.1.2.2) the 4D HF project consisted of a planning phase (years 2012 – 2013) and implementation phase (years 2014 – 2017).

During the study period there was a small, yet significant decrease of the mean age of the study participants, from 80 to 78 years. The share of women was around 46% throughout the period. There were significant increments in proportions of those diagnosed with AF (from 59 to 62%) and HT (from 68 to 79%).

An increase of patient visits to the HF outpatient clinics was seen during the years 2012 to 2017 (from 3 372 to 11 527 visits a year). The increase occurred during the years 2014-2017 when additional economic resources were allocated to the HF outpatient clinics. The number of incoming referrals also increased successively from 2439 in 2015 to 4223 in 2017 (an increase by 73%).

5.2.2 Medication

There were significantly increased percentages of patients receiving both basic and extended HF treatment, with higher increments of drug dispensation among those with previously known HF. These changes are illustrated in Figure 4.

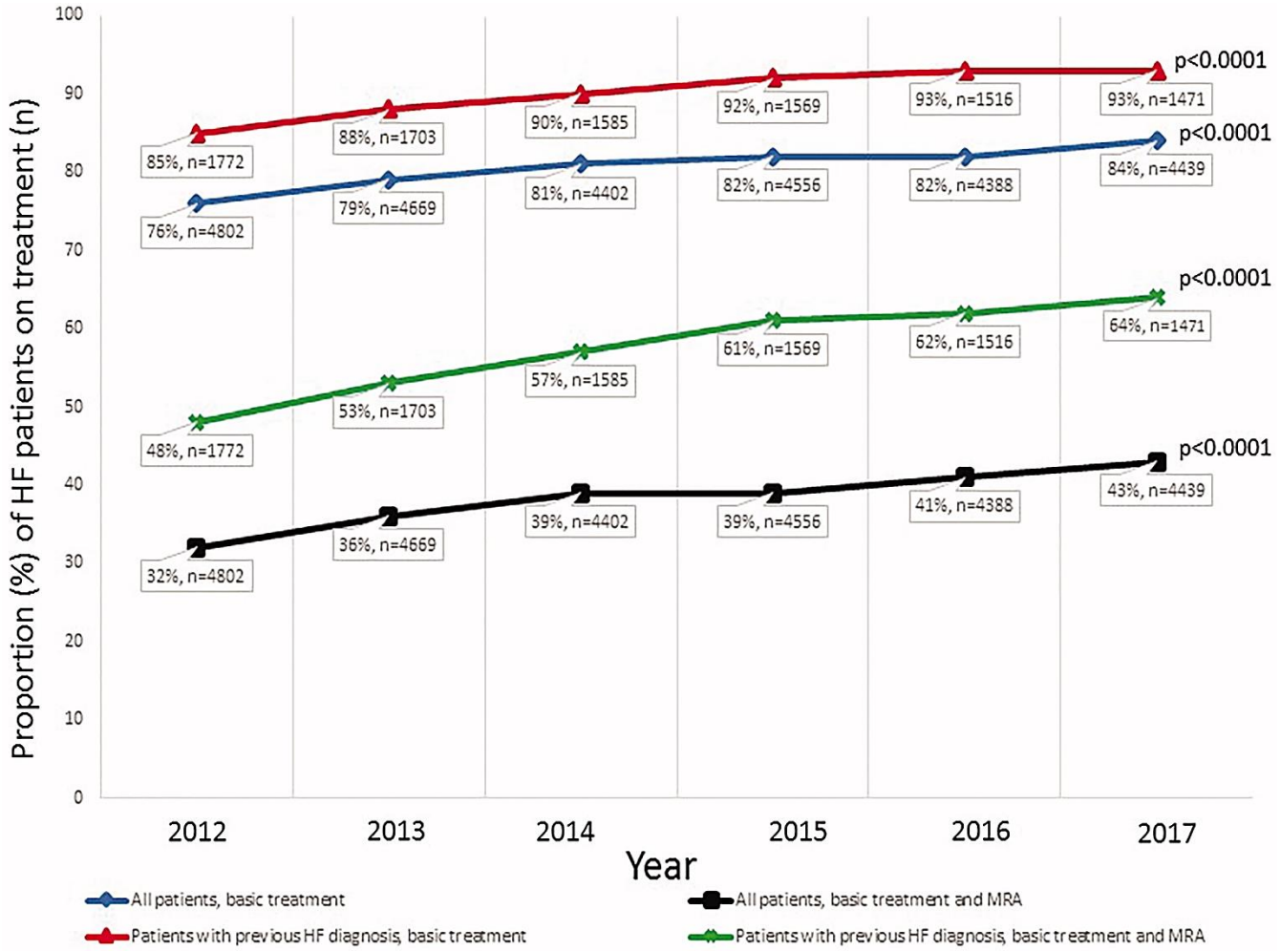

Figure 4. Medication in HF patients in years 2012 – 2017. From: Matan et al. Scand. Cardiovasc. J. 2020.

There were also significant increments in proportions of those treated with OAC.

5.2.3 Outcome

The numbers of HF patients admitted for in-hospital care adjusted per million inhabitants decreased significantly during the study period. The decrement was greater for those who were previously diagnosed with HF. These changes are illustrated in Figure 5.
The combined endpoint of mortality or HF readmission within one year after admission was 48% during the study period. When adjusted for comorbidities and OAC treatment, there was a significant improvement of this outcome by 2% per year (HR 0.98, CI 0.97–0.99, p <0.001), which for 5 years amounts to an improvement by 10% (HR 0.90, CI 0.86-0.95, p <0.001).
After univariate adjustment for OAC treatment, it was shown to be an independent factor associated with a significant improvement of the outcome by 10% over the years 2012-2017 (HR 0.90, CI 0.87–0.93, p <0.001).
One-year mortality of all causes was 19% for the patients without previously known HF, and 26% for those with previous HF diagnosis, which is in line with the findings in a recent Swedish HF registry study [89].

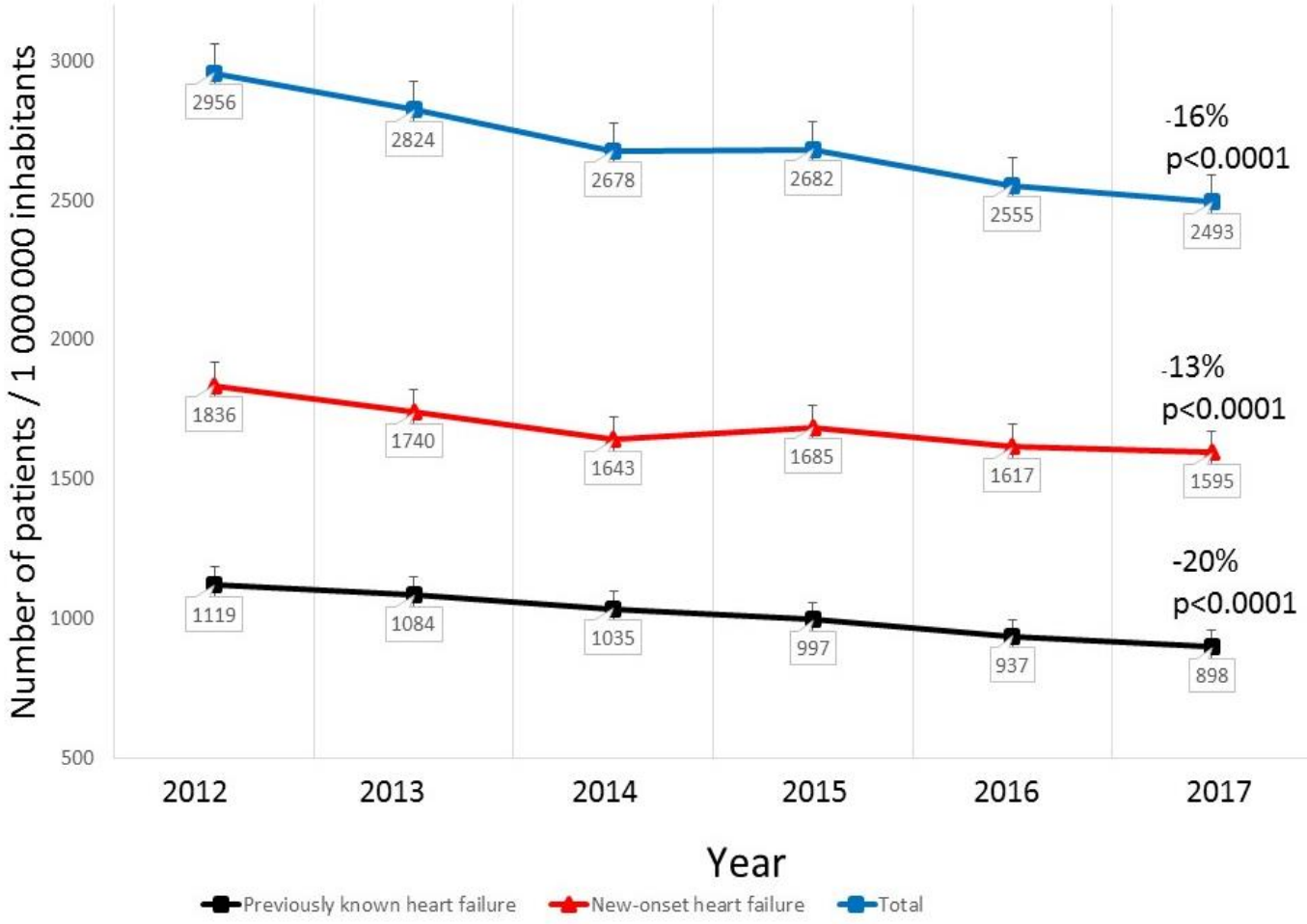

Figure 5. Number of heart failure patients per 1 000 000 inhabitants admitted to 7 emergency hospitals for in-hospital heart failure care 2012 to 2017 in Stockholm, with 95% CI. From: Matan et al. Scand. Cardiovasc. J. 2020.

5.3 PAPER III

5.3.1 For all patients

Levels of EVs exposing Connexin-43/Caveolin-3 and Connexin-43/TnT were higher in coronary sinus compared to radial artery, suggesting that these EVs originated from the heart. The levels of these EVs measured in right atrium were slightly but significantly lower than in samples from the radial artery.
Concentrations of Connexin-43/Caveolin-3 or Connexin-43/TnT were significantly higher in patients with AF compared to those without known AF, while for VE-Cadherin the situation was the opposite.
When measured in coronary sinus, concentrations of EVs exposing Connexin-43/Caveolin-3 correlated significantly with NT-proBNP, LAVI, TR Vmax (maximal flow velocity) and LVEF, and concentrations of EVs exposing Connexin-43/TnT correlated significantly with NT-proBNP, LAVI, LVMI and LVEF.
For VE-Cadherin, there were significantly higher levels measured in coronary sinus compared to radial artery, but there were no correlations to the HF variables.
For MPO and PTX3, there were also slightly but significantly higher levels measured in

coronary sinus compared to radial artery, suggesting that a small amount of MPO-EVs was released into the coronary circulation. However, the levels in right atrium were higher than those in coronary sinus, suggesting a contribution of these EVs from the systemic circulation. For N-Cadherin there were lower levels in coronary sinus than in radial artery, suggesting that these EVs were trapped in the blood vessels of the coronary circulation or in the cardiomyocytes.

5.3.2 After grouping of the patients according to the HF phenotype

For EVs exposing Connexin-43/Caveolin-3 or Connexin-43/TnT there were significantly higher concentrations in coronary sinus compared to those in radial artery in all three patient groups (HFpEF, HFrEF, Normal). The highest transcoronary concentration gradients were measured in the HFrEF group. For EVs exposing Connexin-43/Caveolin-3 the concentration was significantly higher in the HFpEF group than in the Normal group. These findings are illustrated in Figures 6 and 7.
Also for VE-Cadherin and PTX-3 the EV concentrations in coronary sinus were higher than those in radial artery.
For MPO-EVs there were elevated levels in coronary sinus only in the Normal group, and for other sampling locations there were no significant differences.

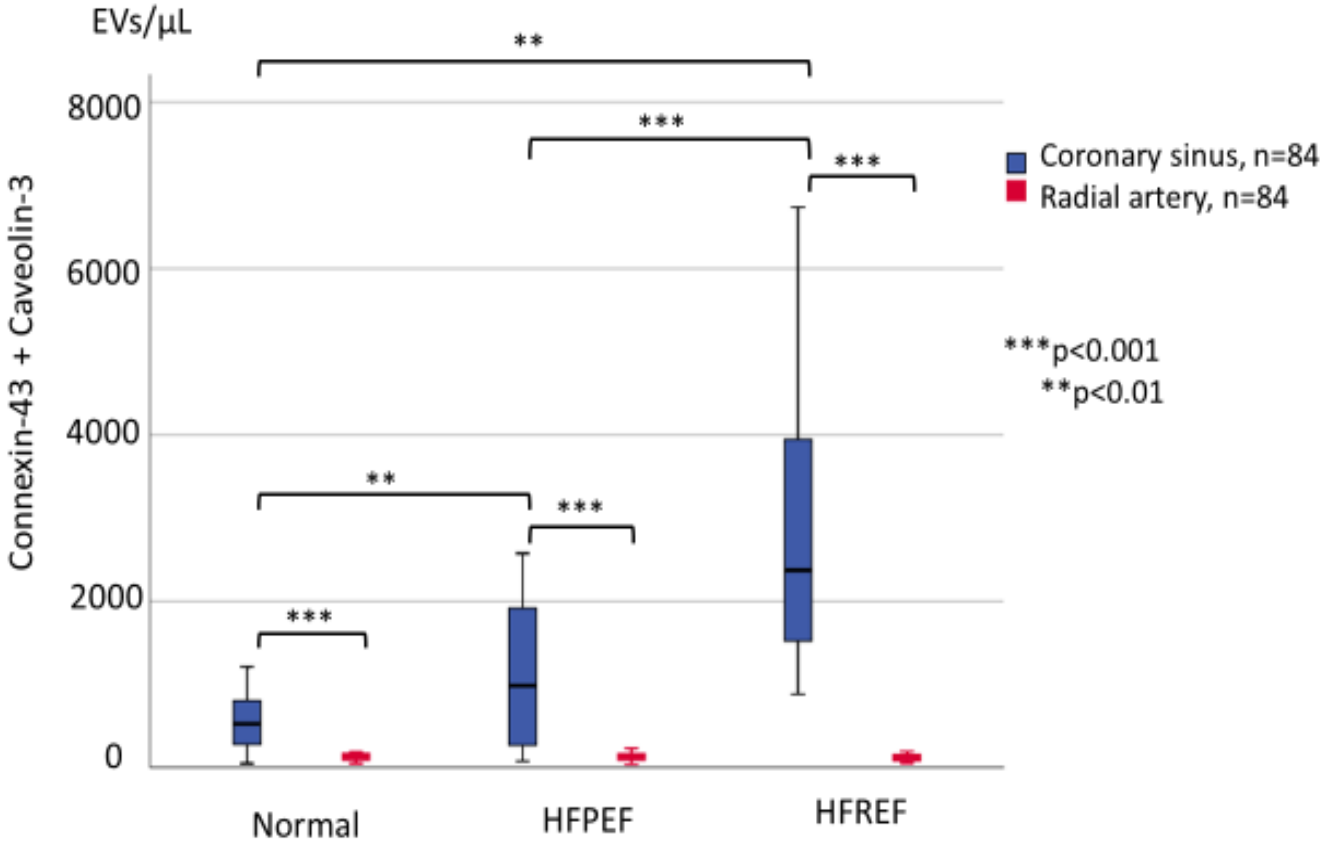

Figure 6. Comparison of the levels of Connexin-43/Caveolin-3 in blood samples taken in the coronary sinus and in the radial artery. The following levels are shown in every box-plot: median, minimum, maximum and the IQR.

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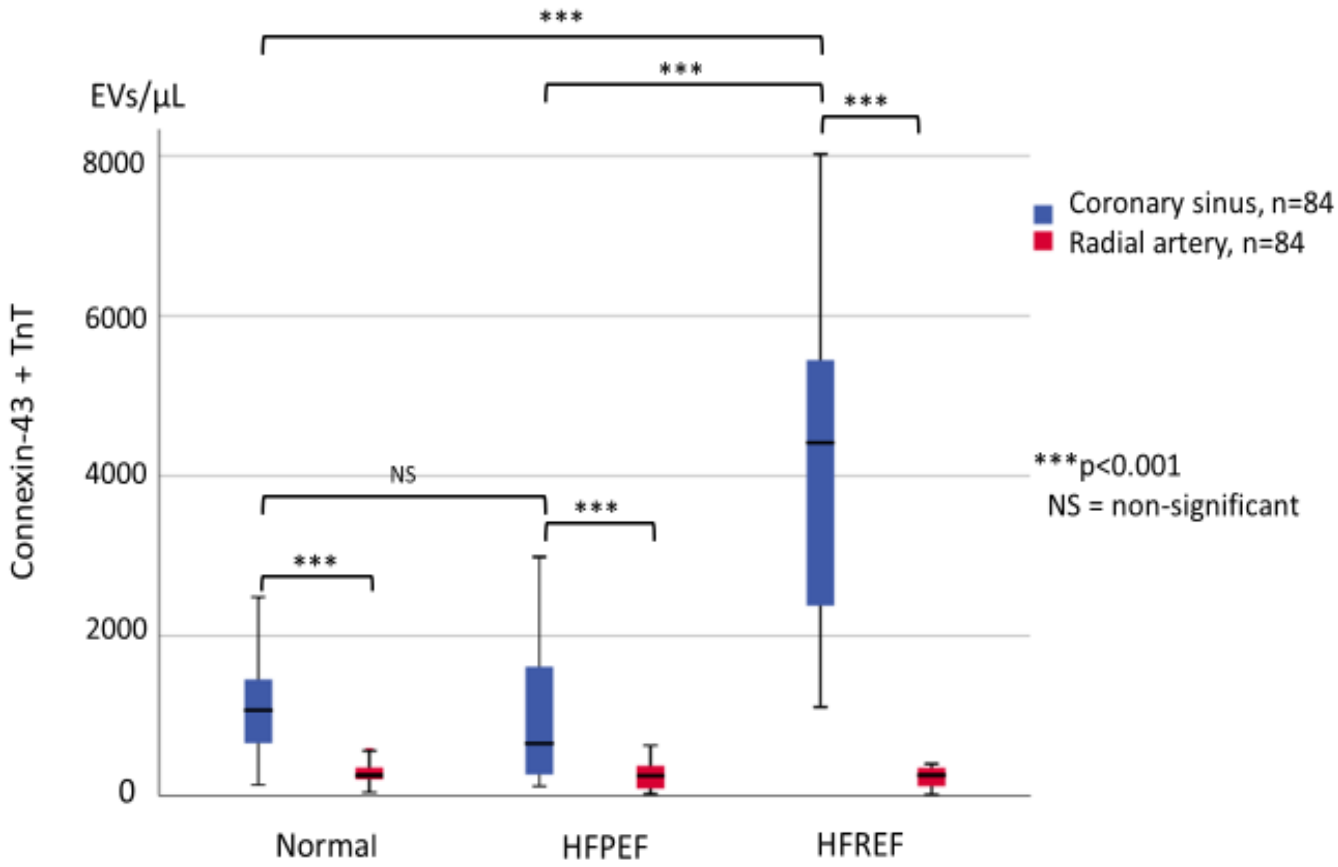

Figure 7. Comparison of the levels of Connexin-43/TnT in blood samples taken in the coronary sinus and in the radial artery. The following levels are shown in every box-plot: median, minimum, maximum and the IQR.

5.4 PAPER IV

5.4.1 Fibrosis biomarkers

There were differences in CITP levels and CITP:MMP-1 values between HFpEF and HFrEF groups, with higher values in the HFpEF group for both biomarkers ($p < 0.05$). There were no differences between the two groups for PICP and MMP-1. The findings are summarized in Figure 8.

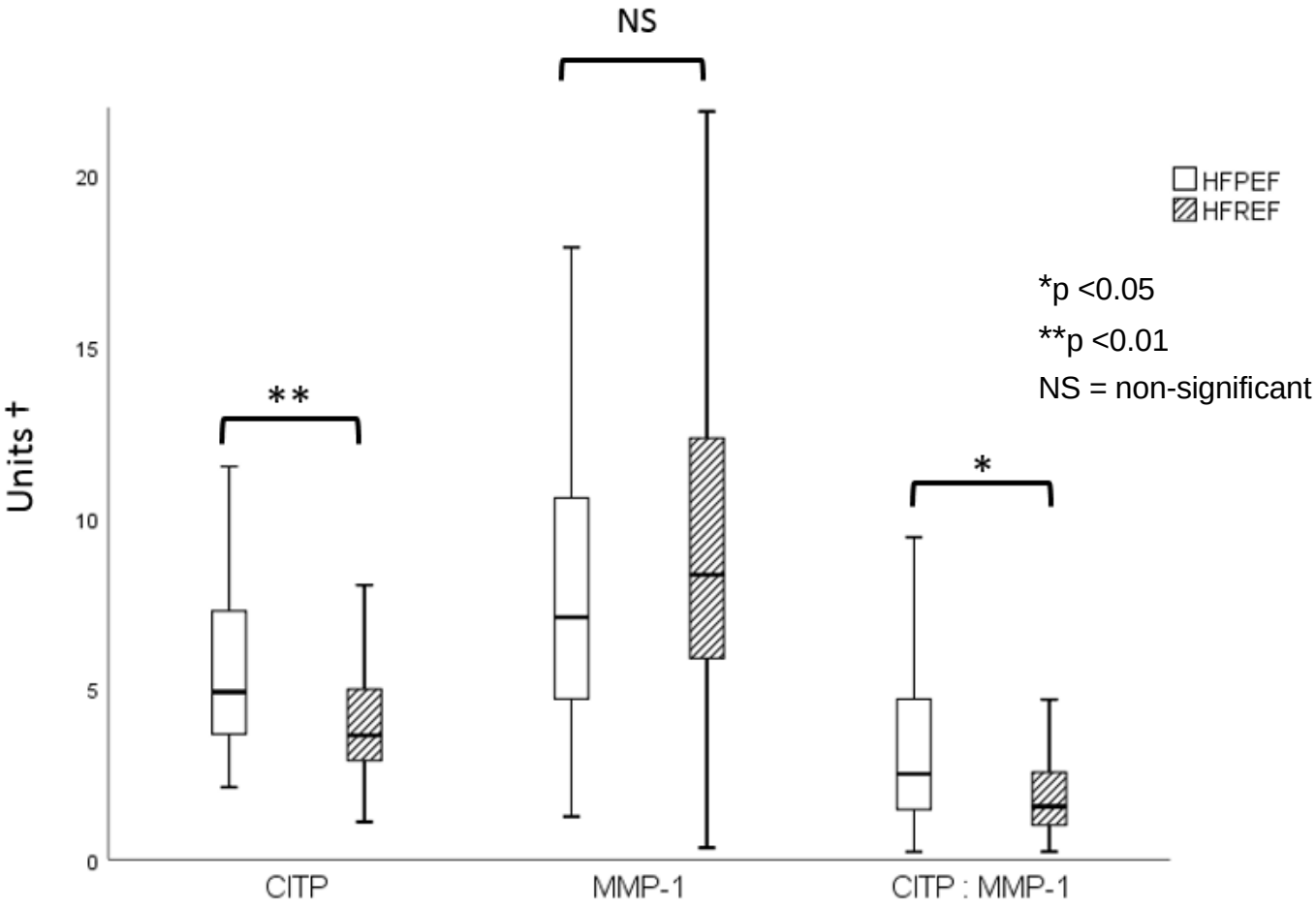

Figure 8. Comparison of the biomarker levels, depending on HF class. The following levels are shown in every box-plot: median, minimum, maximum and the IQR.

Two of the biomarkers (CITP and CITP:MMP-1) were found to correlate with the following parameters: age, NT-proBNP, NYHA-class and eGFR. The correlation was negative for eGFR and positive for the other four parameters. Further, there were correlations between PICP and NT-proBNP, between CITP and LVEF and between CITP and Creatinine.
A univariate logistic regression analysis showed that CITP and CITP:MMP-1 were predictors for increased odds for HFpEF vs HFrEF (OR >1, p <0.05). After a multivariate logistic regression analysis with adjustment for sex, HT, AF and DM, only CITP remained a significant predictor (OR 1.15, CI 1.03 - 1.28, p=0.014).

5.4.2 VCAM-1

The VCAM-1 levels reflecting inflammation were significantly higher in the HFpEF group than in the HFrEF group. There were also correlations between the levels of VCAM-1 and following variables: age, NT-proBNP, NYHA-class, p-creatinine and eGFR.

6 GENERAL DISCUSSION

6.1 PAPER I

6.1.1 Combining clinical diagnosis and echocardiography

In this study we found that a patient population admitted with acute HF and with suspected HFpEF based on Framingham criteria, LVEF ≥45% and mildly elevated BNP or NT-proBNP could later be confirmed in a very high proportion by a comprehensive echocardiography according to current ESC guidelines and analysed in a core laboratory, which in previous studies showed to lead to a high extent of reproducibility [171,172].
In parts of Sweden, as well as in other European countries, echocardiography may not be easily available, which makes HF diagnostics more difficult [173], and the echocardiography reports are phrased in many different ways. Therefore, in Stockholm County an improvement project has taken place, described more in detail in Paper II in this thesis and meant to lead among other things, to a more standardized and comprehensible way to report echocardiography findings [2].

The ESC diagnostic HFpEF criteria state that only one of four criteria need to be met for a HFpEF diagnosis if symptoms and signs and increased natriuretic peptides were found in patients with preserved LVEF. In this case we found that a very high proportion of patients could objectively be diagnosed with HFpEF. However, if both structural LV disease and diastolic dysfunction criteria were to be met still 92% of the patients could be diagnosed with HFpEF. Secondly, the study shows that criteria for diastolic function are possible to classify in almost all available patients. As these parameters have been present in current echocardiographic guidelines and clinical guidelines for several years, we estimate that they are commonly available in most echocardiographic routine protocols, not only in the 4D HF echocardiographic protocol. Our findings strengthen the indication for using echocardiography in HFpEF diagnostics, which is in line with previous studies [174,175].

6.1.2 The role of echocardiography in predicting the outcome

Echocardiography is necessary in HFpEF diagnostics, which has been shown in several studies [174,176,177]. The cut-off values for the echocardiographic parameters were in accordance with the Guidelines and previously performed studies [28,178]. We found it was possible to grade the DD in most patients using a smaller number of echocardiographic parameters, which is in line with the findings in a previous study that was carried out on a similar population and where a core laboratory also was used for analysis of the echocardiographic studies [178]. In this study, we found that two factors were independent predictors of worse outcome: a high number of abnormal echocardiographic parameters and a presence of moderate or severe DD compared to normal and mild DD. These results support use of echocardiographic parameters and cut-off values according to the ESC Guidelines for prognosis of HFpEF patients. Further, results are in line with those of previous studies that investigated use of echocardiography for prognostic purposes in HFpEF, as for example I-PRESERVE [179], TOPCAT [180] and CHARM [178]. A recent study of HFpEF patients showed that an increase of the following echocardiographic parameters could predict adverse outcome: LVMI, E/e´, systolic pressure in pulmonary artery and size of the right ventricle [181]. In another paper from the KaRen study it was shown that using individual echocardiographic variables only E/e´ could be used as an independent prognostic parameter [149] with access to all clinical data. However, the grading model and the ESC diagnostic model used in this study is more in line with the analyses in an everyday clinical situation.

6.1.3 Natriuretic peptides

It is important to use natriuretic peptides when screening for HF [182] because normal levels of these peptides are strong negative diagnostic factors [13]. Further, high levels of BNP or NT-proBNP also carry strong positive predictive value for a true HF diagnosis and they are also strong predictors of adverse cardiovascular outcomes [183,184]. Both natriuretic peptides used in this study, BNP and NT-proBNP, are biomarkers that can be analysed in HFpEF diagnostics [178,185-187], together with echocardiographic variables in accordance with the ESC Guidelines. In the I-PRESERVE study it was showed that NT-proBNP was the most important prognostic parameter for adverse cardiovascular outcomes [188].
In this present study use of echocardiography together with natriuretic peptides contributes to an increased prognostic ability. On the other hand, echocardiography is still essential for diagnosis because LVEF needs to be assessed as well. This notion is in line with the findings of a recent large systematic review comprising 51 different studies on natriuretic peptides and HFpEF [189] and stating that NT-proBNP due to its high negative predictive value can be used for ruling out DD, but for diagnosing HFpEF it must be combined with echocardiography, as the current guidelines recommend.

In HF patients treated with ARNI, it was noted that the BNP-levels rise, making BNP unsuitable for monitoring of the results of the treatment, while the levels of NT-proBNP are unchanged as they are not affected by the inhibition of neprilysin [190].

6.1.4 Patients and clinical signs of HF

To be included in this study, the patient must present with a clinical picture of HF in accordance with the Framingham criteria, because of the importance of these signs for diagnosis and prognosis in HF [44,191], which has been confirmed in several studies [185,192]. In the KaRen study there is no control group to compare our results to. In spite of this, it is very likely that the hospitalised patient population have a high probability of HF even without echocardiography, as it comprises patients with acute onset HF where clinical signs and laboratory analyses of increased natriuretic peptides together add diagnostic specificity for the diagnosis of a HF syndrome. The Framingham criteria have very high sensitivity, but only moderate specificity [193]. The diagnostic accuracy in the study is therefore strengthened by analysis of natriuretic peptides [186] and by clinical HF diagnoses that are well validated in Sweden [194,195]. Thus, the chosen population have a high positive predictive value for a true HF diagnosis, and BNPs are mandatory for HFpEF diagnosis according to the ESC guidelines. Of note, normal values of natriuretic peptides can be found in HFpEF patients, e. g. those with a high body-mass index [196].

6.1.5 Future perspectives - paper I

New echocardiographic methods may be developed and used for HFpEF diagnostics, e.g. diastolic stress-echocardiography or methods for assessing LV strain/ strain rate or left atrial dysfunction [197]. It is also possible that new biomarkers for HFpEF will emerge, e. g. EVs or fibrosis biomarkers, as described in papers III and IV of this thesis.

6.2 PAPER II

6.2.1 HF care organization

The 4D HF project was an extensive one. It followed a whole HF population of a major urban region for several years, which gives the project a high external validity. Its internal validity

is lower, which is described in the Limitations section below. The project was carried out in a series of steps: discussion of the extent of the existing problems with the involved caregivers, presentation of educational material for the primary care, such as an Internet-based program for HF management and a common echocardiography routine for the whole Stockholm County. The capacity of the outpatient HF clinics at the emergency hospitals was increased during 4 years, and the doctors in primary care were encouraged to refer their HF patients to these clinics. Educational efforts were also made towards the patient organizations. Numbers of visits to the HF clinics increased 3-4-fold, which possibly could be attributed to increased awareness of HF among the caregivers as a result of the educational programs on HF that were included in the 4D HF project.
Thus our results indicate that for improvement of HF outcome, it is necessary to combine increased resources of HF clinics with access to cardiological expertise at the hospitals and near co-operation with the primary care and other caregivers. This allows early diagnosis and treatment in society and potentially reduced morbidity and mortality and better quality of life for the patients.
According to a study performed in Sweden, optimization of HF care could lead to an improvement in heart function and reduction of costs and hospital admissions [198].
Other organizational procedures have been reported to improve HF outcome or to change assessment of HF patients. One study showed that use of tele-medicine in structuring of HF management led to a reduction in both hospitalization and mortality [199]. A recent study from UK emphasizes the importance of checking the HF patients of high age for frailty, because it is a significant risk factor for worsening HF and hospital admission [200]. We suggest that such frailty assessments could be made by HF nurses in primary care.

6.2.2 Outcome

We chose to assess prospectívely outcome before and during implementation of the 4D HF program with a combination of all-cause mortality or HF-caused hospital readmission, which after covariate adjustment showed a small annual decrease in risk. This outcome is the primary outcome in most RCT studies of HF because of its importance and relevance for patients, to reduce frequent HF hospitalizations and the high mortality. This finding was independent of the changed burden of comorbidities or increase in OAC medication. These results suggest that an optimized HF care that is more available at outpatient clinics leads to a decreasing need of HF-related hospital admissions. This interpretation is supported by the Swedish National Guidelines for Cardiac Care where in-hospital HF is regarded as possible to avoid, as long as medication and follow-up can be provided in outpatient clinics [135]. The HF treatment given within the project is evidence-based and recommended by the ESC HF Guidelines [13]. The results are in line with the findings in recent randomized trials where structured remote HF management led to better HF treatment and prognosis [199,201]. A recent study from the UK also showed a significant correlation between adherence to a national HF handling program and lower risk for HF-related readmissions [202]. It is important to add that our results are not conclusive as our program is not an RCT study and the results only show an association with improved outcome over time.

6.2.3 Patients

The mean age of the patients decreased during the project time. Further, there was a decline of the proportion of patients with IHD, which may have been caused by improved treatment and prevention [203].

As described above (chapter 5.2.1) there was an increase of patients with AF and HT during the years. These diagnoses are common comorbidities of HFpEF [204,205]. This finding may be explained by a better awareness of HFpEF in the primary care, leading to an increased

referral of these patients to the HF open-patient clinics. Recently, increased efforts have been made to diagnose asymptomatic AF, e. g. with thumb ECG [206,207].

6.2.4 Medication

According to the ESC Guidelines patients with HFrEF should be treated with RAASi and BB, and in patients who have symptoms and LVEF ≤ 35% MRA should be added [13]. In this study we have seen an increase of dispensation of HF drugs in patients discharged from hospitals. Patients who were previously diagnosed with HF were more often treated with more extensive medication, and they had a greater decrement in hospital admissions, compared to those with new-onset HF. This finding could possibly be explained by the intervention within this project.
The results of the 4D project (84-93% of the HF patients treated with RAASi and BB) suggest an improved compliance with the current HF guidelines, following the objectives outlined in of the Swedish national guidelines of cardiac care [135]. These results can be compared to the numbers stated in a recent Swedish epidemiological study [208] where between 52 and 74% of HF patients were treated with RAASi. According to the report from the Swedish National Board of Health and Welfare (https://www.socialstyrelsen.se/statistik-och-data/oppna-jamforelser/) for years 2017-2018, 73% of the patients in Sweden were treated with RAASi and BB within 6 months after being hospitalized with a HF diagnosis, which is lower than the results seen in the 4D project.

The number of patients who received treatment with OAC increased. This finding reflects the fact that prescription of OAC to patients diagnosed with AF in the Stockholm County increased during the last years [209]. In patients treated with OAC an improvement of outcome was seen comparable to the improvement over time seen with the 4D HF project, which possibly could be explained by diminished incidence of stroke due to OAC [210]. Treatment with OAC in HF patients with AF are recommended by the ESC HF Guidelines [13]. A high proportion of the patients in our study had AF. It is known that there is a correlation between the severity of HF and the AF prevalence [211], therefore a high percentage of AF in our study may be explained by the fact that it included patients with severe HF that more often require hospitalization, which is also indicated by the severe one-year prognosis.

6.2.5 Future perspectives – paper II

A follow-up study would be needed in a near future to evaluate if the project had long-term effects, preferably with a comparison with another urban area in Sweden where such a project has not been carried out. It would also be of value to study how the health care handled decisions on the continuation of the expanded resources in the light of the positive findings during the project.

6.3 PAPER III

6.3.1 Cardiomyocyte-derived EVs

We found that concentrations of EVs exposing Connexin-43, which is a gap junction molecule that is expressed on cardiomyocytes [212], and Caveolin-3 or TnT, which both are present in cardiomyocytes [154,213] were 5-7 times higher in blood samples collected from coronary sinus compared samples from radial artery. Thus, we conclude that these EVs are of

cardiomyocyte origin. Further, we found that the transcoronary gradients of these EVs were much higher in the HFrEF group, compared to the HFpEF or the Normal groups. In HFpEF Connexin-43/Caveolin-3-EVs were elevated with an around 7-fold elevation over the heart, which was significantly higher than in patients with normal diastolic function. Caveolin-3 is present in the caveolae of cell membranes of myocytes [154], and experimental and animal studies have shown that it may be involved in "cell wound repair" [153], and also in the development of cardiomyopathy [214,215]. In our study, we used flow cytometry with antibodies towards both Caveolin-3 and Connexin-43. The EVs exposing Caveolin-3 and Connexin-43 showed significant correlations with both preoperative NT-proBNP levels in plasma as well as echocardiographic variables such as LAVI, TR maximal velocity and LVEF. These observations suggest that circulating EVs co-exposing Caveolin-3 and Connexin-43 reflect an ongoing process involved in HF pathophysiology.

EVs exposing Connexin-43 together with TnT did also show a significant transcoronary concentration gradient, with higher levels in the HFrEF group than in HFpEF or Normal. The levels of Connexin43/TnT EVs in coronary sinus were, however, similar in HFpEF and Normal. Furthermore, the levels of Connexin-43/TnT EVs in coronary sinus correlated significantly with preoperative NT-proBNP levels, and LAVI and LVEF, similar to what was observed also for the Connexin-43/Caveolin-3 EVs. To the best of our knowledge, the presence of TnT on circulating EVs has not been observed before, although it has been proposed [213]. It may be speculated that TnT exposed on the EVs are derived from cytosolic troponin pools, where the troponin molecules through hitherto unknown mechanisms are bound on the EV membrane during the EV-formation process, and then carried out in the circulation. This finding, together with elevated EVs exposing Connexin-43/Caveolin-3 could reflect and ongoing injury – cell repair process within the failing myocardium. This seemingly pathological process is more pronounced in HFrEF compared to HFpEF, which in gross could reflect the fact that HFrEF in most studies is associated with a more severe prognosis than HFpEF. We can, however, not be certain about what mechanisms that lead to generation of these cardiomyocyte derived EVs, whether it is due to apoptosis or an ongoing reparative process in the cardiomyocytes, due to some underlying pathophysiology. Myocardial ischemia caused by the surgery may be discussed as an explanation, but in our case the blood sampling was performed directly after sternotomy and before start of the cardiopulmonary bypass, which should minimize the risk of ongoing cardiac ischemia, but this possibility cannot be ruled out. Whatever the underlying mechanisms can be, cardiomyocyte EVs deserves to be further investigated in the context of HF, as well as in other heart diseases.

6.3.2 VE-Cadherin EVs

For VE-Cadherin we noticed significantly higher EV concentrations in coronary sinus than in the radial artery, in all three patient groups. However, there were no significant differences in VE-cadherin EV concentrations between these groups, and no correlations between EVs exposing VE-Cadherin and different HF-related variables. VE-Cadherin exposing EVs do likely not reflect HF or its severity or phenotype, but more likely reflect the severity of concomitant atherosclerotic disease in the vasculature including the coronary arteries [81,82], and perhaps also a functional impairment of the vascular endothelium, as supported by the previously described strong correlation between circulating VE-Cadherin EVs and endothelial dysfunction [80].

6.3.3 Other EVs

Regarding PTX3, which appears to be at least partly produced in the coronary vasculature [216] we found significant transcoronary gradients within all three phenotypes, but no significant differences between groups in the absolute concentrations in the coronary sinus samples. Our interpretation of these findings is that the elevated concentration of PTX-3 exposing EVs could be explained by an inflammation in the vasculature, in line with a pre-existing IHD. Another finding was that the EV concentrations in right atrium were higher than those in in coronary sinus, suggesting that the systemic circulation contributes significantly to circulating EV levels. For MPO and N-Cadherin we observed data that were similar to those for PTX3. The contribution of these EVs in our study is likely the effect of an inflammatory state with activation of leukocytes, partly caused by ongoing surgery. N-Cadherin EV concentrations in coronary sinus were lower than those in radial artery, suggesting that these EVs partly accumulated when passing the heart.

6.3.4 Summary

In this hypothesis-generating study, after assessing transcoronary gradients of EVs, we found EVs originating from the myocardium, with higher EV concentrations measured in patients with HFrEF than in those with HFpEF or patients without HF. For EVs exposing Connexin-43/Caveolin-3 the concentration was significantly higher in the HFpEF group than in the Normal group. These cardiomyocyte-derived EVs showed significant relationships to HF-related clinical variables. Measurements of cardiomyocyte derived EVs can potentially give a better understanding of the pathophysiological processes that take place in the cardiac muscle in HF.

6.3.5 Future perspectives – paper III

Additional studies are warranted to confirm the findings in this hypothesis-generating study and to explore whether the cardiomyocyte-derived EVs could be used as biomarkers for diagnosing and perhaps even phenotyping HF. There is also a need for further studies in HF where EVs are measured in peripheral venous blood, as the "experimental set-up" used in this study (blood sampling from central locations, and additionally performed during coronary surgery) is invasive and requires central catheterization, and thus not suitable for everyday practice. We need tools which better help us understand HF and to perform HF phenotyping, as we know that an impairment of both systolic and diastolic LV function can be sub-clinical and yet lead to increased mortality [217], while evidence-based treatment leads to an improved prognosis [94,218]. Biomarkers which can be used in phenotyping can aid in performing precision medicine, i.e. selecting the right HF treatment for the right HF patient.

6.4 PAPER IV

6.4.1 Fibrosis biomarkers

We found that CITP was an independent biomarker for new-onset HFpEF vs HFrEF. Previously, it has been shown that higher levels of CITP and CITP:MMP-1 indicate increased collagen turnover and reduced collagen cross-linking. It is also known that there is an association between HFpEF and LV hypertrophy with increased extent of cardiac fibrosis [64,219], while HFrEF is associated with increased degradation of collagen, with a correlation between CITP-levels and NYHA class [66]. Therefore, our findings are surprising and suggest that new-onset HFpEF patients, compared to those with HFrEF, have an increased turnover of myocardial collagen type 1. It could possibly be explained by the fact that the patients in our study all have new-onset symptomatic HF without long term HF

medications. We can speculate that collagen degradation in a small and hypertrophic heart typical of HFpEF could benefit hemodynamically by a lowering of LV end-diastolic pressure through this degradation.

CITP levels were associated with AF, HT and DM, all three of which are common in HFpEF [204,220], with presence of these comorbidities resulting in higher CITP levels. All three conditions are known to be associated with cardiac fibrosis [221-223]. Our findings therefore support previous hypothesis that these prevalent comorbidities may drive HFpEF also by an increased turnover of collagen in the myocardium.

In our study, we found that both CITP and CITP:MMP-1 had a negative correlation with eGFR. A recent study showed that CKD is the most frequent non-cardiac comorbidity in HFpEF [224]. Several multifactorial mechanisms have been proposed to explain this comorbidity, including altered hemodynamics, increased inflammatory state and immune-mediated processes [225]. Another study suggested that CKD could facilitate development of cardiac fibrosis, assessable by measurement of the levels of biomarkers, such as PICP and CITP:MMP-1 [226]. Thus, our findings are consistent with previous notion that worsened HF correlates with declined kidney function.

Finally, there is an uncertainty whether our measurements of fibrosis biomarkers in the venous blood do reflect conditions in the myocardium. As mentioned above (section 2.6.1) these biomarkers can also be affected by collagen metabolism in other organ systems, e.g. the bone tissue. However, the correlations that were found between the levels of the biomarkers and both natriuretic peptides and echocardiographic parameters of HF suggest a possible cardiac origin of the biomarkers in this study.

There are other biomarkers for cardiac fibrosis, not studied in this thesis, such as sST2, galectin-3 and collagen III N-terminal propeptide, that were shown to correlate with the levels of NT-proBNP and with echocardiographic parameters such as E/e´ and LAVI [68], which probably makes them possible to be used to diagnose HF.

The recently completed PARAGON HF trial showed that the levels of fibrosis biomarkers were altered favourably by the ARNI treatment compared to ARB treatment in a way suggesting diminished building of myocardial fibrosis; it was also shown that these biomarkers could be used for prognostic assessment in HFpEF [227]. This provides additional support to the notion that fibrosis is an important part of HFpEF pathogenesis.

6.4.2 VCAM-1

The role of VCAM-1 in the pathogenesis of HFpEF has not been investigated much previously. Our finding that VCAM-1 levels are higher in the HFpEF group as compared to HFrEF is in line with the previous notion that inflammation, in particular microvascular dysfunction plays a role in the pathogenesis of HFpEF [50]. There was also a recent study on HFpEF in human and animal models, showing that empagliflozin, a SGLT2-inhibitor, could reduce inflammation and oxidative stress, lowering the levels of VCAM-1, ICAM-1, TNFa and IL-6, and also reducing stiffness of the cardiomyocytes [228]. In a recently presented randomized controlled trial, dapagliflozin led to an improvement of the diastolic function of LV in patients with diabetes [229]. Together, these data support the hypothesis that inflammatory mechanisms are important in the pathogenesis of diastolic dysfunction.

6.4.3 Future perspectives – paper IV

Additional studies are warranted to explore in what extent the fibrosis biomarkers can serve for diagnosing and/ or phenotyping HF. These studies should prospectively compare new-onset and chronic HF, and also include a control group of individuals with no HF.
A larger study on the PREFERS material is now ongoing, including 546 participants and 60 controls, as outlined in the design paper [2,230].

7 LIMITATIONS

7.1 PAPER I

Firstly, this study does not include an age-matched control group without HF, which makes it difficult to compare echo-parameters between healthy persons and those with HFpEF. However, it is possible to compare our findings to the normal reference values of echo-parameters from the NORRE study [231], which is the largest European registry study in Europe performed by EACVI.
Secondly, although it is known that the echocardiographic parameters used in diagnosis of DD vary with age [232], it is not accounted for in this study. However, the results from a previous study performed by this group [178] show that adjustment for age of these echo-parameters does not significantly affect their prognostic value, nor does it change the proportions of patients in different sub-groups of DD within the same group of HFpEF patients [233].
Thirdly, in this study one of the inclusion criteria was LVEF ≥45% instead of >50% which is recommended by the current ESC guidelines [13]. However, there were only nine patients with EF ≤50%, and they were kept in the analyses because they belong to the new HF category presented in guidelines, namely HFmrEF, which is diagnosed using the same objective criteria as those used in this study.
Fourthly, the core lab echocardiography was not performed immediately after the acute presentation, but 4-8 weeks later. However, our opinion is that probability of significant changes of the echocardiographic parameters during this short period of time is low.

Further, the ESC Guidelines and Consensus Paper recommend several echocardiographic parameters for assessment of structural and/ or functional changes in the LV for diagnosing HFpEF [13,234]. The more abnormal signs can be found, the more support is there for a correct HFpEF diagnosis. Our results support this algorithm, as we have found that a high number of abnormal echocardiographic parameters (≥4) add diagnostic value to HFpEF diagnosis as the risk for methodological error for a single variable thereby is reduced.

7.2 PAPER II

Firstly, it is not known how many of the patients have HFpEF as the echocardiographic data are not available. It lowers the internal validity of the project. In the nested research study of HF (PREFERS) where a similar population in Stockholm County is examined, which is performed in the Stockholm County and investigating a similar HF population, the HFpEF prevalence is 44% (25% EF >50% and 19% EF 40-50% - unpublished data), which corresponds with the numbers in other epidemiological HF studies [235]. Further, in this project all patients admitted to hospitals in the Stockholm County with HF as first- and second-position diagnosis were included, which increases the external validity of the project.
Secondly, there is no evidence that the above-mentioned basic treatment (BB and RAASi) are effective in treatment of HFpEF [13]. Nevertheless, there is a high prevalence of various comorbidities in HFpEF, such as HT and IHD that constitute an indication for the basic

treatment. Therefore, we have reason to believe that the basic treatment was correctly used in the majority of HFpEF patients.
Thirdly, it is not known how high percentage of the patients reached the defined target doses of the basic medication. However, data on the prescriptions that were dispensed in the pharmacies is included in the study. The SIGNAL-HF study for example showed that structured management of HF leads to a higher proportion of patients who reached target doses [133]. Further, we know that achievement of target doses is a very important part of the work performed in the HF clinics during this project, which enables us to assume that a higher percentage of the patients receive target doses as a result of this project.

7.3 PAPER III

Firstly, there is an uncertainty whether the study participants diagnosed with HF really have HF symptoms, as proxy diagnoses for HF are used in this study instead of clinical diagnoses. As mentioned above (section 2.5.1), IHD is less usual in HFpEF patients: about 1/3 of the patients that develop HF as a complication of myocardial infarction have HFpEF [236,237]. In our study material all patients had IHD, which has led to their CABG surgery. However, in our opinion this weakness is compensated by the fact that we had the possibility to study EV concentrations in the central circulation, which made it possible to better understand the pathophysiological processes in the heart.
Secondly, all the patients had ischemic heart disease, which was the reason for their CABG surgery. This is not the case with a typical HF population, even though IHD is a common cause of HF [13]. However, this approach allowed us to measure transcoronary concentration gradients of EVs, which was very important for this hypothesis-generating study aimed at understanding the roles of different EVs for the pathophysiology of HF.

7.4 PAPER IV

Firstly, this is a hypothesis-generating pilot study, and therefore incomplete and it does not include a follow-up or a control group of patients without HF. However, the larger final PREFERS study with 546 participants is soon to be completed and analyse. It will include a 12 month follow-up, as stated in the PREFERS design paper [2], and also a control group of 60 individuals.
Secondly, the distribution of study participants in our study is not typical for the general HF population, as women are in minority. However, in our HFpEF group the majority of the patients were women. In a study of >8500 patients with new-onset HF from 2013, women were in majority (51%), and female gender was a strong predictive factor for HFpEF [238].
Thirdly, in this study the patients with HFpEF are in minority. However, this is the case in many other HF studies. As patients with HF with mid-range EF (HFmrEF) usually constitute a large group [239], the size of the HFpEF group is dependent on the cut-off values for LVEF. In this study we chose to have only two HF groups according to the design of the PREFERS study [2].
Fourthly, this study does not include myocardial biopsies which is regarded to be a “golden standard” for diagnosing myocardial fibrosis. However, the levels of the biomarkers of fibrosis have been shown to have a good correlation to the amount of myocardial fibrosis which makes them to a non-invasive substitute for biopsy [60].

8 CONCLUSIONS

8.1 PAPERS I - IV

In Paper I, we could show that relatively simple diagnostic methods as clinical signs of HF, a slight elevation of natriuretic peptides and LVEF ≥45% later confirmed by a new echocardiography analysed at a core laboratory were sufficient for correct HFpEF diagnosis in a large majority of the patients followed up after hospitalization for suspected HFpEF. Secondly, based on our results, the ESC HF Guidelines can be used for assessment of the prognosis in patients with HFpEF.

In Paper II, we could show that a multidisciplinary creation of a uniform program for management of HF care in manifold expanded hospital-based HF clinics introduced in the Stockholm region, including educational measures towards primary care and hospital care givers were associated with an increased use of HF medication, reduced numbers of HF hospitalizations and a reduction in the combined outcome of this study (all-cause mortality within one year or HF-related hospital readmission).

In Paper III, we found a transcoronary gradient of some EVs exposing myocyte-specific proteins (Connexin-43, Caveolin-3 and TnT), which suggests that these EVs originate from cardiomyocytes. This gradient is higher in patients with HF, especially in the HFrEF group.

Finally, in Paper IV we could see that in patients with new-onset HF, there was a higher degree of collagen degrading and reduced collagen-crosslinking in the HFpEF group, compared to the HFrEF group. Furthermore, CITP may be possible to use as a biomarker for differentiation between new-onset HFpEF and HFrEF, and secondly, CITP is associated with risk indicators for HF outcome, such as NT-proBNP, AF, HT and DM, and E/e´.

In this thesis several different aspects of heart failure are investigated. Our results show that the diagnostical algorithm for HFpEF proposed by the ESC seem valid and useful. Further, we study the effects of improved HF management in a large urban area and find that a better outcome for the patients can be suggested by this care program. Finally, we study different novel blood-borne biomarkers that potentially can be used in clinical practice for better understanding of the pathogenesis of HF, and especially HFpEF, and potentially may serve as future drug targets in HFpEF where there presently is no evidence-based treatment available.

8.2 CONCLUDING REMARKS

In this thesis we show the importance of systematic implementation of current HF guidelines for diagnosing and treating HF. We also show the importance of optimizing HF management in a greater urban area for positive patient outcomes.
HF is a common name for a clinical syndrome with a variety of phenotypes and of different pathogenesis. HFpEF is a phenotype with poor prognosis and without evidence-based treatment. Novel biomarkers may provide new information about the pathogenesis and prognosis of HF, and lead to improved characterization of HF phenotypes including HFpEF. Further studies of HF in the complete range of HF phenotypes using the new biomarkers investigated in the present thesis are warranted, including long-term follow-up in relation to remodelling of LV function, and long-term patient outcome.

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